Title: NC 03699 Pesticide Residues in Tobacco, Tobacco Products, and Main-Stream Smoke

Project Leader: T. J. Sheets

I. Summary of Research:

Effect of Sprinkler Irrigation on MH Residues and Sucker Control. An overhead irrigation of 0.26 cm at 3 and 6 hr after MH application and a heavy irrigation of 2.16 cm at 3, 6, and 9 hr after MH application reduced MH residues and sucker control on flue-cured tobacco. The heavy irrigation affected residues and sucker control more than the light irrigation. The light irrigation at 9 hr and the heavy irrigation at 12 hr also reduced MH residues but not sucker control. Reapplication of a 0.25X rate (0.62 kg/ha) of MH did not restore sucker control lost due to the heavy irrigation, but reapplication of the 0.5X rate (1.25 kg/ha) did. Reduced MH residues were associated with "directed" sprays during reapplication of the 0.5X rate.

Disappearance of Prime+ Residues from Flue-Cured Tobacco. A field experiment was established at the Border Belt Tobacco Research Station, Whiteville, and at the Central Crops Research Station, Clayton, and Prime+ was applied at 0, 1.2, and 2.4 lb/acre (0, 91, and 182 mg/plant) in four replications. Yields and sucker control will be reported by H. Seltmann. Green tips were sampled at 0, 1, 2, and 4 days after application and were stored at -20° C. Samples of cured tobacco from all harvests at both locations were collected, dried, and ground in preparation for analysis. A method of analysis is presently being evaluated for use with these samples. The analyses will be carried out as soon as the method is found to be satisfactory.

EBDC and ETU Residues in Burley and Flue-Cured Tobacco. A separate method of analysis was developed for EBDC and ETU residues. EBDC was determined by electron capture gas-chromatography, and ETU by HPLC with UV detection. Samples of burley from 1983 field experiments conducted by Dr. P. B. Shoemaker at Laurel Springs and Waynesville were analyzed for both residue components. In one experiment EBDC residues averaged 54, 88, and 103 ppm and ETU residues averaged 0.13, 0.16, and 0.17 ppm for leaf from lower, middle, and upper stalk positions, respectively, when mancozeb (2 lb/100 gal) was applied at 5-day intervals. With 14-day intervals in another test, residues were much lower (<0.05 to 25 ppm). A field residue study was conducted jointly with Dr. C. E. Main at Clayton in 1984. When mancozeb (2 lb/100 gal) was applied every 7 days from late May to early July, residues of EBDC for the four harvests were 23.0, 14.2, 9.5, and <0.05 ppm. With a 14-day interval, residues were lower. ETU residues were detected only in fourth harvest samples at 0.07 to 0.10 ppm. Samples of burley from Dr. Shoemaker's 1984 experiment at Waynesville are now being analyzed.

Insecticide Residues in 1983 and 1984 Tobacco Products. Average levels of total DDT residue (TDE + DDT) in 1983 cigars, pipe tobacco, cigarettes, snuff, and chewing tobacco were 2.27, 0.41, 0.26, 0.20, and 0.18 ppm,

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respectively. The average concentration in each product dropped slightly in 1984 with corresponding values at 1.95, 0.22, 0.14, <0.12, and 0.16 ppm. Averages for toxaphene and dieldrin were below the lowest detectable limit for all products in both years. Endrin residues average 0.05 ppm for cigars both years and 0.02 ppm or below for all other products.

MH Residues in 1983 Flue-Cured and Burley Tobacco. MH residues in composite samples of flue-cured tobacco varied from 29 to 292 ppm over all belts and stalk positions. Middle Belt tobacco had the highest average residue (173 ppm) and Old Belt tobacco the lowest (109 ppm). The average over all belts and stalk positions was 138 ppm. The MH residue for burley samples from all marketing areas and three stalk positions was 102 ppm. Average residues were highest for Area C (145 ppm) and lowest for Area D (52 ppm).

MH Residues in 1984 Tobacco Products. Average MH residues in US cigarettes increased from 50 ppm in 1981 to 59 ppm in 1983 and 66 ppm in 1984. Residues in other products remained at about the level as in 1981 and 1983. Average concentrations for cigarettes, pipe tobacco, snuff, chewing tobacco, and cigars were 66, 27, 26, 11, and <10 ppm, respectively.

Free and Bound MH. Analyses of samples from tobacco treated in the field in 1983 with MH were completed in 1984. MH residues in green leaves taken 24 hours after application varied with the time of day that MH was applied. Concentrations of both free and bound MH were greater when MH was applied at 10:00 a.m. and 2:00 p.m. and lowest when applications were made at 2:00 and 6:00 a.m. For applications at 6:00 and 10:00 p.m., residues were intermediate. Sucker control was positively correlated with MH residue. In a greenhouse study, the bound MH fraction in green leaf tissue increased with time after application but represented only 3.3% of total residue 24 hours after treatment. Absorbed but free MH also increased with time and at 24 hours after application represented about 35% of the total residue (surface + free absorbed + bound residue).

II. Graduate Students:

Meyer, Susan A., M.S., Toxicology
Thesis title: Free and Bound MH in Flue-Cured Tobacco as Affected by Time of Spraying

Requirements for the degree to be completed by August 1985.

Atwood, Stanley T., M.S., Toxicology
Thesis title: Stability of Pesticides in Spray Tanks

Requirements for the degree to be completed by August 1985.

V. Manuscripts Accepted for Publication:

Hunt, T. W., G. W. Wojeck, and T. J. Sheets. Exposure of applicators to maleic hydrazide (MH). Bull. Environ. Contam. Toxicol. (in press).

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Herman, N. D., T. W. Hunt, and T. J. Sheets. Exposure of hand harvesters to maleic hydrazide (MH) in the field. Bull. Environ. Contam. Toxicol. (in press).

Sheets, T. J. and H. Seltmann. Residue and sucker control from two formulations of MH. Beitr. Tabakforsch. (in press).

Sheets, T. J., R. B. Leidy, and W. J. Mistric. Residues of methiocarb in flue-cured and burley tobacco. Tob. Sci. (in press).

VI. Manuscripts in Review:

Leidy, R. B., T. J. Sheets, and L. A. Nelson. Residues of fluvalinate and permethrin on flue-cured tobacco. Beitr. Tabakforsch.

VII. Papers Presented at Professional Meetings:

Harrell, B. H., Jr. and T. J. Sheets. Persistence of low levels of picloram in some North Carolina soils and its effect on flue-cured tobacco. Presented by B. H. Harrell at the meeting of the Sou. Weed Sci. Soc. Hot Springs, AR. Jan. 1984.

IX. Acknowledgements:

This work was supported in part by grants from the North Carolina Tobacco Foundation, the American Tobacco Company, Brown and Williamson Tobacco Corporation, Liggett & Myers Tobacco Co., P. Lorillard Corporation, Philip Morris, Incorporated, R. J. Reynolds Tobacco Company, and the Tobacco Advisory Committee (United Kingdom).

Appreciation is expressed to the following individuals for their cooperation during 1984:

W. R. Baker, Jr.
S. T. Barnes
F. G. Bond
N. M. Chopra
G. B. Clark
W. C. Clements
R. D. Coltrain
Joy M. Fields
H. O. Gentry
H. A. Hardison
Janet S. Hayes
W. L. Jones
E. P. Lampert
J. W. Laws
R. B. Leidy
C. E. Main
Patricia L. Messick
L. A. Nelson
L. N. Newman
N. T. Powell
Ernest Price
H. Seltmann
P. B. Shoemaker
W. H. Ward
W. W. Weeks

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TOBACCO GENETICS

3504 GENETIC INVESTIGATIONS IN NICOTIANA SPECIES POPULATIONS

E. A. Wernsman and D. F. Matzinger

I. Summary of Research:

A. Genetic Modification of Total Particulate Matter

Studies have been conducted to evaluate the ability to select within a tobacco population to modify genetically the delivery of total particulate matter (TPM) in tobacco smoke. Estimates of genetic and environmental variances and covariances in a Black Shank Synthetic population indicated that sufficient genetic variability existed for TPM to provide for genetic modification of the trait. However, a positive genetic correlation between TPM and total alkaloids (TA) suggested that selection for lower TPM would lead to correlated decreases in TA.

Because of difficulties in obtaining a large number of TPM analyses from small samples of tobacco, the PMI procedure was used to initiate selection. Five cycles of recurrent mass selection were conducted for lower PMI. A companion study was also conducted using a restricted selection index involving both PMI and TA in an attempt to select against the correlation between these two traits. Seven cycles of index selection were conducted.

An evaluation of the five cycles of selection for low PMI and the seven cycles of index selection was conducted at two locations with eight replications at each location. PMI, TA, and sugar data are presented in Table 1. In the low PMI study, PMI was reduced from 220.6 to 203.1 mg/g in five cycles of selection with a correlated decrease in leaf TA from 2.45 to 1.59%. Since TA was decreasing at a faster rate than PMI, the PMI/TA ratio increased. Very little change was observed in reducing sugar content. In the index study, there was a slight increase in PMI from 213.9 to 225.3 mg/g in seven cycles of selection. However, the increase in leaf TA from 2.45 to 3.11% led to a decrease in the PMI/TA ratio. With the emphasis on maintaining TA, reducing sugars decreased since TA and reducing sugars are negatively correlated.

Agronomic data were also obtained from these experiments to evaluate any correlated changes associated with low PMI or index selection (Table 2). The most noticeable change appears to be a moderate decrease in yield in both selection studies.

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Table 1. Mean response in low PMI and index selection populations.

Cycles	PMI mg/g	TA %	PMI/TA	Sugars %
C0	220.6	2.45	8.99	17.5
C1	223.6	2.27	9.87	17.5
C2	214.8	2.32	9.28	16.8
C3	214.3	2.28	9.39	17.7
C4	213.8	1.90	11.23	18.7
C5	203.1	1.59	12.74	18.5
Ix C1	213.9	2.45	8.72	17.1
Ix C2	214.7	2.47	8.68	17.7
Ix C3	219.2	2.70	8.11	16.2
Ix C4	222.8	2.75	8.11	17.2
Ix C5	218.2	2.92	7.48	16.8
Ix C6	219.6	2.97	7.40	15.6
Ix C7	225.3	3.11	7.25	15.2
LSD .05	7.4	0.21	0.99	1.3
.01	9.7	0.27	1.30	1.7

Table 2. Mean performance of agronomic characters in low PMI and index selection populations.

Cycles	Yld. #/A	Grade Index	Flower days	Hght. cm	Lvs. no.	Lgth. cm	Wdth. cm
C0	2751	51.6	52.4	128	18.0	61.4	33.8
C1	2604	51.1	51.7	126	16.9	60.8	33.9
C2	2647	49.1	52.5	127	17.7	57.6	34.9
C3	2652	49.6	50.8	126	17.6	59.9	32.2
C4	2589	50.4	51.5	131	17.3	60.3	33.1
C5	2508	48.1	51.5	136	17.1	59.6	32.7
Ix C1	2589	49.5	51.8	129	17.5	59.4	33.1
Ix C2	2719	51.9	53.0	129	17.3	60.9	33.8
Ix C3	2546	50.5	52.4	131	17.1	59.9	34.0
Ix C4	2386	50.7	51.7	129	17.3	58.4	33.5
Ix C5	2466	51.7	50.9	126	16.6	58.4	33.0
Ix C6	2243	52.1	51.4	128	17.3	57.5	32.8
Ix C7	2394	51.3	51.5	125	16.8	59.0	32.9
LSD .05	100	NS	1.0	4	0.6	1.3	1.1
.01	132	NS	1.3	5	NS	1.7	NS

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Average selection responses were determined as the regression of cycle means on the cycle number. These regressions were estimated for the characters subjected to selection to measure the direct response to selection and for all other traits evaluated to measure changes in any correlated characters. Regression estimates for both selection experiments are presented in Table 3. In addition to the intercept (a) and the average response (b), the average change per cycle is expressed as a percentage of the source population from which selection was initiated (b/C0).

Table 3. Average selection response per cycle for selected and correlated traits.

	Low PMI Sel.			Index Sel.		
	a	b	b/C0	a	b	b/C0
PMI, mg/g	223.5	-3.4*	-1.5	213.7	1.4*	0.6
TA, %	2.52	-0.15*	-6.1	2.32	0.11*	4.9
PMI/TA	8.61	0.66*	7.6	9.03	-0.27*	-3.0
Sugars, %	17.1	0.27	1.5	17.4	-0.24	-1.4
Yield, lb/A	2714	-36*	-1.3	2682	-53*	-2.0
Grade Index	51.3	-0.54	-1.0	49.7	0.34*	0.7
Flower, days	52.2	-0.20	-0.4	52.6	-0.19	-0.4
Height, cm	125.0	1.64	1.3	129.3	-0.38	-0.3
Leaves, no.	17.6	-0.08	-0.4	17.6	-0.10*	-0.6
L. Lgth., cm	60.5	-0.25	-0.4	59.3	-0.10	-0.2
L. Wdth., cm	34.2	-0.31	-0.9	34.3	-0.22*	-0.6

*Significantly different from 0 at P = 0.05

In the low PMI selection study, PMI was reduced an average of 3.4 mg/g/cycle or a reduction of 1.5% of the mean per cycle. The correlated decrease in leaf TA was 0.15% per cycle, over 6% of the mean. The more rapid decrease of TA compared with PMI led to an average increase of PMI/TA of 0.66 or 7.6% of the mean. With index selection there was a slight increase in PMI of 1.4 mg/g/cycle or 0.6% of the mean. However, leaf TA increased at a much faster rate, 0.11%/cycle or 4.9% of the mean. The relatively small change in PMI compared with the rapid increase in TA led to an average reduction of PMI/TA of 0.27 or 3% of the mean. In seven cycles of index selection, the PMI/TA ratio has been reduced over 20%.

B. Evaluation of the Mitochondrial Genome (with M. M. Bland)

Little is known about the structure and organization of plant mitochondria DNA (mtDNA). Unlike animal mitochondrial genomes which are typically 15 to 18 kb in length, plant mitochondria contain unusually large and complex genomes, varying in size from 250 to 2500 kb. This variation in size among plant mitochondrial DNAs is not understood. The mitochondrial genome of

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tobacco is estimated to be approximately 250 kb and is composed of heterogeneous classes of circular molecules.

Mitochondrial DNAs from Nicotiana tabacum, an amphiploid, and its putative progenitor species, N. sylvestris and N. tomentosiformis were compared in structure and organization. Restriction endonuclease digests of mtDNA from these three species showed a complex pattern of approximately 40 bands using PstI, to over 50 bands using HindIII. The BamHI and PstI restriction patterns of N. tabacum and N. sylvestris appeared nearly identical, while the HindIII pattern showed additional bands in N. sylvestris of about 6.7 and 3.0 kb and the absence of a 3.4-kb fragment in N. sylvestris. Restriction patterns of N. tomentosiformis mtDNA obtained with BamHI, HindIII and PstI digestions showed a complex array of fragments that bear little similarity in migration with those of the other two species.

Mitochondrial genomes of Nicotiana species are large and sufficiently complex that it is difficult to follow organizational changes by analyzing the positions of visible restriction fragments. The use of hybridization to detect homologous sequences offers a more precise method of studying mtDNA variation in these species. Accordingly, six clones of mtDNA containing either defined genes or regions known to be transcribed were hybridized with restriction endonuclease fragments of mtDNA from the three Nicotiana species.

The results indicate that the mitochondrial DNA of N. tabacum was inherited from N. sylvestris. Conservation in organization and sequence homology between mtDNAs of N. tabacum and the maternal progenitor, N. sylvestris, provide evidence that the mitochondrial genome in these species is evolutionarily stable. Approximately one-third of the probed restriction fragments of N. tomentosiformis mtDNA showed conservation of position with the other two species. Pattern variations indicate that extensive rearrangement of mtDNA has occurred in the evolution of these Nicotiana species.

C. Investigations of Haploid Methodologies for Tobacco Improvement

Studies were conducted to define the magnitude of genetic changes resulting from the use of the N. africana technique for haploid production and the in vitro midvein tissue culture method to achieve chromosome doubling. A doubled-haploid breeding line (first cycle maternal doubled haploid, Ky 10 1C MDH1) of burley cultivar Ky 10 was produced by the N. africana technique and chromosome doubled by in vitro leaf midvein culture and was crossed as the female parent with pollen of N. africana. Haploid plants from this cross were identified, chromosome doubled, and self-pollinated. Cultivar Ky 10, a doubled haploid composite composed of equal numbers of seeds of 10 first cycle MDH lines, Ky 10 1C MDH1, and 16 second cycle MDH lines derived from Ky 10 1C MDH1 were grown in 5 replications of a RCB at Clayton, North Carolina in 1984. Data were collected on green and cured leaf yields, days to flower, number of leaves per plant, plant height, leaf length and width, and percentage of nicotine in the cured leaf. Cultivar Ky 10 yielded 8% more green leaf, flowered four days earlier and was 9 cm shorter than the MDH materials. Second cycle MDH lines differed from their MDH parent only for plant height. However, genetic variability was present among the second cycle lines for days to flower, plant height and leaf length and width. Although these genetic

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changes were statistically significant, they were very small; yielding ability of the second cycle MDH lines had not changed.

Second cycle MDH lines from first cycle MDH parents from cultivars C139 and NC 95 were grown at Whiteville and Kinston, North Carolina, in 1984 and compared with their parents and the original cultivars. Results similar to those observed with the burley germplasm were obtained. These studies demonstrate that the N. africana method combined with the in vitro midvein chromosome doubling technique is a viable means of producing homozygous tobacco genotypes in a single generation, and that these lines are agronomically competitive with genotypes developed by conventional inbreeding. The method should shorten time requirements for cultivar development.

D. Anther Culture-Induced Changes as a Source of Variability for Tobacco Improvement (with R. J. Schnell II)

The potential value of anther culture-induced somaclonal variability for tobacco, Nicotiana tabacum L., breeding was investigated. Sixty-four cytologically verified chromosome-doubled haploids (DHs) from a single plant of a highly inbred cultivar, 'NC95', were chosen at random. The DHs were crossed in a North Carolina factorial mating Design II of Comstock and Robinson (1952). Four males and four females were randomly assigned to each of eight sets, and the 16 full-sib families within each set, 128 full-sib families total, were grown and evaluated in a randomized complete block design at Kinston, North Carolina in 1981 and 1982. The NC95 DH full-sib families differed from NC95 for yield, plant height, and percentages of reducing sugars and nicotine in the cured leaf. Significant variability existed among DH full-sib families for all characters measured except leaf number. Mean leaf yield of the NC95 DH full-sibs was 12% below NC95 and not a single full-sib family equaled NC95. Genetic variability generated in the DH population was additive and estimates of dominance variance were negligible.

The two best yielding full-sibs, and the parental DH line giving the highest yielding half-sib family within sets were selected and intercrossed. Equal quantities of seed from each cross were bulked to produce populations C_1 FS and C_1 HS, respectively. Populations C_0, C_1 FS, C_1 HS, a DH composite and two sublines of NC95 were evaluated in two environments. The selection techniques were effective in achieving improved yields, and populations C_1 FS and C_1 HS were 5.8% and 4.9%, respectively, higher yielding than population C_0. Based on estimates of additive genetic and phenotypic variances, three cycles of full-sib family selection would be required to produce a population of equal yielding ability as cultivar NC95.

Four sets of the design II progenies were screened for their reaction to Phytophtora parasitica va. nicotianae race 0. Although NC95 is highly resistant to tobacco black shank, susceptibility to the fungus occurs in field plantings of some of the DH progeny. Increased susceptibility was evident in greenhouse screenings; however, increased resistance was also detected in some full-sib families as well. Significant estimates of additive and dominance genetic variances were found among NC95 DH full-sib families for the disease reaction. Negative correlations existed between the disease index and all agronomic and leaf chemical characters except percent reducing sugars in the

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cured leaf. These results suggest that genetic changes for increased resistance as well as increased susceptibility were resulting from anther culture in this material.

The black shank susceptible cultivar '402' was used for haploid isolation and 130 DH families were produced from four 402 plants. The 130 DH families were screened in the greenhouse in three experiments for the disease reaction. Selections were made in each experiment based on highest disease index (most resistant families); these selected families were intercrossed and seed bulked from each mating. Selected and unselected populations and selected DHs were then evaluated in greenhouse disease evaluations. No increase in resistance among the selected 402 DHs or in the selected 402 DH population was observed. It is concluded that anther culture of the highly susceptible cultivar 402 did not result in genetic variability for increased resistance.

E. Evaluation of Varieties and F_1 Hybrids Differing in Their Blue Mold Disease Reaction (with Rebeca Rufty)

Resistance to Peronospora tabacina has not been observed in the germplasm collection of tobacco, Nicotiana tabacum. Programs to induce mutations conferring disease resistance or transfers of genetic mechanisms for disease resistance from alien Nicotiana species must exhibit dominance or partial dominance, and the character must be qualitatively inherited. Three flue-cured cultivars with some resistance to P. tabacina (Ovens 62, Sirone and Sirogo) have been released from the Australian blue mold breeding program. The genetic mechanism for disease resistance in these cultivars should exhibit some degree of dominance.

In North Carolina environments, Ovens 62, Sirone, and Sirogo exhibit poor plant form, produce abundant suckers, and appear to be low yielding. Nevertheless, Ovens 62 and the F_1 hybrid of Ovens 62 with susceptible American cultivar, McNair 944, have exhibited some resistance to this disease in blue mold nursery plantings at Gurabo, Puerto Rico. Any commercial utilization of such hybrids would require cytoplasmic male-sterile females as parents for efficient hybrid seed production. An objective of the 1984 program was to evaluate the agronomic performance and leaf chemical constituents of normal and cytoplasmic male-sterile (cms) F_1 hybrids of the Australian cultivars with popular American varieties.

Four American cultivars, McNair 944, Coker 347, Speight G-28, and Speight G-70, were converted to cms. The N. suaveolens source of cms was utilized, since 1983 results at Gurabo, Puerto Rico, indicated that this cytoplasm had no effect on blue mold disease reaction. Each Australian cultivar, Ovens 62, Sirone, and Sirogo was crossed to the normal (fertile) and cms (sterile) form of each American cultivar. The three Australian cultivars, the four American cultivars in fertile and cms condition, and the sterile and fertile F_1 hybrids of each Australian cultivar crossed with each American cultivar were grown and evaluated in 1984 at Whiteville and Kinston, North Carolina. The 35 genotypes were grown in three replications of a randomized complete block design with 20 competitive plants per plot at the two locations. Entries were evaluated for leaf yields, leaf quality as measured by grade index, and percentages of total alkaloids as nicotine and reducing sugars in the cured leaf. Cured leaf

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of Ovens 62, cms Speight G-70 x Ovens 62, cms Speight G-70, cms McNair 944 x Ovens 62 and cms McNair 944 were submitted to R. J. Reynolds Tobacco Company for physical evaluation, cigarette manufacture, and smoke panel evaluation.

Male-sterile and male-fertile cultivars and hybrids did not differ from each other for any parameter measured. This suggests that the N. suaveolens cytoplasm had no differential effect on cultivar or hybrid performance, and that the genotypes of the American cultivars had been adequately recovered in their conversion to cms. The Australian cultivars, as expected, were significantly lower yielding than American cultivars and the F_1 hybrids, but their cured leaf quality was equal or superior to the American varieties. Furthermore, they possessed a more "balanced" leaf chemistry in terms of total alkaloids as nicotine, reducing sugars, and sugar-nicotine ratios (Table 4). F_1 hybrids were intermediate between the parents in agronomic performance and chemical composition, and were superior to the American cultivars in physical aspects of leaf quality. Results of smoke panel evaluations are unavailable at this time.

Although these results are preliminary, they suggest that F_1 hybrids of American cultivars with Ovens 62 could be considered as an "emergency" measure for blue mold control if a Ridomil-resistant strain of P. tabacina were to arise. In particular, the F_1 hybrid of McNair 944 x Ovens 62 was intermediate in plant form, produced 3149 kg/ha cured leaf, had a grade index of 50, 2.21% total alkaloids and 18.8% reducing sugars. This performance was competitive with that of the American cultivars in this test (Table 4).

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Table 4. Performance of Australian blue mold-resistant cultivars, susceptible American cultivars, and their F_1 hybrids at Whiteville and Kinston, North Carolina, in 1984.

Genotype	Yield kg/ha	Grade Index	Reducing Sugars %	Total Alkaloids %
Ovens 62	2374	49	15.3	2.77
Sirone	2241	48	14.5	2.61
Sirogo	2594	49	16.3	2.43
Mean, Australian Cultivars	2403	49	15.4	2.60
McNair 944[1]	3278	47	17.2	2.22
Coker 347	3281	47	17.6	2.23
Speight G-28	3088	48	18.1	1.89
Speight G-70	3268	51	18.3	2.53
Mean, American Cultivars	3229	48	17.8	2.22
American cvs x Ovens 62[2]	3085	51	17.3	2.24
American cvs x Sirone	2985	47	17.7	2.29
American cvs x Sirogo	2700	48	18.4	2.28
Mean, All Hybrids	2924	49	17.8	2.27

[1] Mean of American cultivars in normal and cms forms.
[2] Mean of the four F_1 hybrids with American cultivars in normal and cms forms.

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II. Graduate Students:

Bland, Molly M., Ph.D. Candidate, Genetics. Molecular genetics of tobacco.

Corbin, Thomas C., Ph.D. Candidate, Crop Science. A comprehensive tobacco improvement program utilizing parthenogenic haploids.

Witherspoon, William David, Jr., Ph.D. Candidate, Crop Science. Utilization of the haploid sporophyte as the selection unit in plant breeding.

Yung, Carl H., Ph.D. Candidate, Crop Science. DNA amplification in anther culture-derived haploids and its relationship to changes in tobacco metabolism.

III. Postdoctoral Fellows:

Murphy, S. Kathryn. Cloning of Nicotiana DNA.

IV. Publications:

Beatson, R. A., E. A. Wernsman and R. C. Long. 1984. Divergent mass selection for carotenoids in a flue-cured tobacco population. Crop Sci. 24: 67-71.

Bowman, D. T., E. A. Wernsman, T. C. Corbin and A. G. Tart. 1984. Contribution of genetics and production technology to long-term yield and quality gains in flue-cured tobacco. Tob. Sci. 28: 30-35.

Matzinger, D. F. and L. G. Burk. 1984. Cytoplasmic modification by anther culture in Nicotiana tabacum L. J. Hered. 75: 167-170.

Matzinger, D. F., W. W. Weeks and E. A. Wernsman. 1984. Genetic modification of total particulate matter. Recent Adv. Tob. Sci. 10: 15-51.

Rufty, R. C., E. A. Wernsman and G. V. Gooding, Jr. 1984. Method for the storage of Peronospora tabacina sporangiospores. Phytopathology 74: 852 (Abstract).

V. Manuscripts Accepted for Publication:

Bland, M. M., D. F. Matzinger and C. S. Levings III. Comparison of the mitochondrial genome of Nicotiana tabacum with its progenitor species. (Accepted by Theoretical and Applied Genetics).

Cockerham, C. C. and D. F. Matzinger. Selection response based on selfed progenies. (Accepted by Crop Science).

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Gooding, G. V., Jr., E. A. Wernsman and Rebeca C. Rufty. Reaction of Nicotiana tabacum L. cv. Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus, and Peronospora tabacina. (Accepted by Tobacco Science).

VII. Papers Presented at Professional Meetings:

Bland, M. M., D. F. Matzinger and C. S. Levings III. Comparison of the mitochondrial genome of Nicotiana tabacum with its progenitor species. 31st Tobacco Workers Conference. Pinehurst, NC. Jan. 1985.

Matzinger, D. F., W. W. Weeks and E. A. Wernsman. Genetic modification of tobacco particulate matter. Eighth International Tobacco Scientific Congress. Vienna, Austria. Oct. 1984.

Matzinger, D. F., W. W. Weeks and E. A. Wernsman. Genetic modification of total particulate matter. 38th Tobacco Chemists Research Conference. Atlanta, GA. Nov. 1984.

Rufty, R. C., G. V. Gooding, Jr. and E. A. Wernsman. Association between resistance to potato virus Y and susceptibility to Peronospora tabacina in tobacco (Agron. Abstracts p. 86). American Society of Agronomy Annual Meetings. Las Vegas, NV. Dec. 1984.

Rufty, R. C., E. A. Wernsman and G. V. Gooding, Jr. Inheritance of resistance to tobacco blue mold. 31st Tobacco Workers Conference. Pinehurst, NC. Jan. 1985.

Weeks, W. E. and D. F. Matzinger. Quantitative changes in steam volatiles from genetically modified flue-cured tobaccos. 38th Tobacco Chemists Research Conference. Atlanta, GA. Nov. 1984.

Wernsman, E. A. Genetic basis for unexpected genetic variability among anther-derived doubled haploids. Minnesota Symposium "Molecular Biology and Its Relationship to Plant Breeding." St. Paul, MN. June 1984.

Wernsman, E. A., D. F. Matzinger and R. C. Rufty. Characterization of androgenic and gynogenic doubled haploids from inbred cultivars. Eighth International Tobacco Scientific Congress. Vienna, Austria. Oct. 1984.

Wernsman, E. A. and R. C. Rufty. Genetic information in different haploid nuclei of inbred plants. 31st Tobacco Workers Conference. Pinehurst, NC. Jan. 1985.

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Kernsman, E. A. and B. W. Smeeton. Factors affecting maternal haploid frequencies in Nicotiana tabacum L. Eighth International Tobacco Scientific Congress. Vienna, Austria. Oct. 1984.

III. Graduate Student Theses Completed During Reporting Period:

Bland, M. M. M.S. Comparison of the mitochondrial genome of Nicotiana tabacum with its progenitor species. 1984.

Schnell, R. J. II. Ph.D. Anther culture-induced changes as a source of variability for tobacco improvement. 1984.

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Title: NC03896 Cell Culture Techniques for the Development of Disease Resistance in Tobacco

Project Leader: Margaret E. Daub

I. Summary of Research:

This project has three broad objectives:

1. To investigate the potential of somaclonal variation for generating novel, disease-resistant genotypes.

2. To develop protoplast fusion techniques for transferring naturally occurring resistance genes from wild Nicotiana species into tobacco cultivars.

3. To investigate the molecular mechanism of resistance in one host-pathogen system (the tobacco/Cercospora nicotianae system) in order to identify genes for resistance which may be important for tobacco improvement.

A. Somaclonal Variation. One of the most promising areas in the use of tissue culture for the development of novel crop genotypes is the observation that the process of growing cells in culture can generate stable, heritable variation which is useful in crop improvement. This variation has been termed somaclonal variation, and has been demonstrated in a number of crops including sugar cane, potatoes, and tomatoes, and for a number of different traits including both disease resistance and agronomic traits. The purpose of this work is to investigate the usefulness of somaclonal variation for developing high quality tobacco cultivars with increased levels of disease resistance.

We have isolated mesophyll protoplasts from two tobacco cultivars (C319 and NC2326) which have desirable quality characteristics but low levels of resistance to several important diseases of tobacco. These protoplasts were induced to form callus, and were maintained as callus for several months in order to increase the probability of inducing variability. Plants were then regenerated from these calli, and transferred to soil in the greenhouse. These plants were allowed to flower, and selfed seed was collected from each plant to be analyzed in the field. This latter step is required in order to ensure that all variation seen among the plants is limited to changes which are passed through a seed cycle.

Seed from protoplast-derived plants are being tested in the field in 1985 for resistance to black shank and for variability in yield and quality characteristics. Resistance to black shank will be tested by planting the seedlings in two black shank nurseries and making bi-weekly stand counts of surviving plants. Lines which show increased disease resistance will be harvested and tested again the following year. In addition to black shank, we are also working to establish a Granville wilt nursery at the Lower Coastal Plain Research Station at Kinston. This nursery will be used to

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analyze our protoplast-derived lines for wilt resistance.

B. Transfer of Resistance Genes from Nicotiana repanda to Nicotiana tabacum by protoplast fusion. A project has been initiated to use protoplast fusion techniques to transfer disease-resistance genes from a wild Nicotiana species, N. repanda into high quality cultivars of flue-cured tobacco. N. repanda is of critical importance because it provides an alternative source of resistance to tobacco mosaic virus, and it carries resistance to two root knot nematode species for which we currently have no other sources of resistance. At the present time, genes from N. repanda have not been incorporated into tobacco cultivars due to the difficulty in crossing N. repanda and N. tabacum by conventional methods. The goal of this work is to use protoplast fusion to generate hybrid germplasm which can serve as a valuable and readily accessible source of resistance genes for a tobacco breeding program.

The general methods which we are using are as follows: protoplasts are being isolated from leaves of N. repanda plants and from leaves of albino plants of cultivated flue-cured varieties (NC 2326 and C 319) grown in vitro. Due to the semi-dominant albino mutation present in the flue-cured cultivars, fusions between these protoplasts give rise to light green plants as compared to the green or albino plants produced by the parents. This allows us to identify our hybrid fusion products in culture. The hybrids can then be transferred to soil and tested for resistance in greenhouse tests.

In the last year we have made progress in the development of techniques for the isolation and fusion of N. repanda and albino N. tabacum protoplasts. We have determined that high yields of healthy albino protoplasts can best be obtained from sterile plants growing in vitro rather than from cell cultures. The best N. repanda protoplasts are isolated from plants growing in growth chambers under 30° day/26° night temperatures and short days. We were unsuccessful in obtaining N. repanda protoplasts from in vitro-grown plants, and greenhouse-grown plants gave variable results. Chemical fusion with polyethylene glycol (PEG) results in approximately 10-20% fusion, and does not appear to harm the protoplasts; fusion with high Ca^{++}/high pH is not effective, and the use of both PEG and high Ca^{++}/high pH is toxic to the cells. At this time we have analyzed approximately 3000 regenerated plants resulting from PEG fusion. Out of these, most are parental types (albino and green). We have approximately 6 colonies that appear at this time to be hybrid fusion products out of an expected 100. This low frequency was not unexpected; previous studies on hybridizations between N. repanda and N. tabacum have demonstrated incompatibility at early stages. To overcome this problem we are 1) experimenting with various culture regimes to try and stabilize the hybrid prior to regeneration, 2) using anther culture to generate N. repanda haploids which may allow for gene introgression without as many incompatibility problems, and 3) using protoplasts derived from an N. repanda - N. sylvestris hybrid which may allow for greater chromosome homology. In addition, we have recently obtained a Zimmerman electrofusion apparatus which will allow us to fuse protoplasts electrically. This procedure allows for higher fusion frequencies than obtained through conventional chemical fusion, and can be manipulated to increase the proportion of cross fusions. We hope that this technology will allow us to

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increase the number of hybrid fusions we are able to generate and thus increase the probability of recovering a hybrid plant.

C. Mechanisms of resistance to the phytotoxin cercosporin produced by members of the genus Cercospora. Cercospora species are a group of plant pathogenic fungi which cause serious losses worldwide on a large number of host species including tobacco, banana, sugar beet, peanut, coffee, rice, and corn. Cercospora species are an interesting group of plant pathogens, not only because of the vast number and diversity of hosts which they attack, but also because they have been very hard to control due to the difficulty in identifying adequate levels of resistance in natural populations of host plants. One reason for the success of this group of pathogens appears to be their ability to produce the extremely toxic, free-radical-generating phytotoxin, cercosporin.

Cercosporin is a photosensitizing compound which is activated by light and then reacts with O_2 to produce singlet oxygen and superoxide. In the plant cell, cercosporin damages the cell membranes by causing a peroxidation of the membrane lipids. The resulting changes in the structure and composition of the plant cell membranes make them very leaky, and it is hypothesized that this provides the pathogen (which is limited to growth within the intercellular spaces of the leaf) with the nutrients required for growth and sporulation in the host. At this time, no sources of resistance to cercosporin in plants have been identified. All plants which have been tested (including those which carry some resistance to the pathogen) are sensitive to cercosporin, and we have been unable to select cercosporin-resistant tobacco and sugar beet cells or protoplasts in culture. Mice and bacteria are also sensitive to cercosporin. The fungus Cercospora and a large number of other fungi, however, are highly resistant to cercosporin and to another singlet oxygen-generating sensitizer, hematoporphyrin. Considering the extreme toxicity of singlet oxygen, the generalized resistance of fungi is surprising, and gives us a unique opportunity to investigate resistance mechanisms, not only to this very damaging phytotoxin, but also to free-radical-generating agents in general.

The purpose of this work is to determine the genetic and biochemical basis of resistance to cercosporin. Since we have not been able to identify or induce cercosporin-resistance in plants, we are using the naturally resistant fungus as our test system. We are initially looking at the role of carotenoids and the fungal cell wall in resistance. We have determined that Cercospora nicotianae and other Cercospora species produce high concentrations (approximately 20 µg/gram dry weight) of carotenoids at early stages of cell growth. β-carotene, a potent singlet oxygen quencher, accounts for approximately 99% of the carotenoids present. We are currently investigating the effects of fungal carotenoid inhibitors on resistance of the fungus to cercosporin. Furthermore, we have been able to correlate the production of carotenoids by other fungi with increased resistance to cercosporin, suggesting that carotenoids play an important role in fungal protection.

We are also investigating the role of the fungal cell wall in resistance. For this study we are using protoplasts of C. nicotianae. The methods which

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we have developed for protoplast isolation are as follows: the fungus is grown for 24 hrs. in shake culture and then digested for 1.5 hrs. with an enzyme solution containing β-glucuronidase and cellulase. Cercospora protoplasts regenerate walls and form visible colonies in 3-4 days. We have found that C. nicotianae protoplasts are more sensitive than the fungal mycelium to cercosporin, but are still considerably more resistant than plant tissue. Only approximately 50% of freshly isolated C. nicotianae protoplasts are killed by exposure to 1 μM cercosporin (a concentration which is lethal to plant cells). By four hours, all protoplasts have regained their resistance. At 10 μM cercosporin (a concentration which does not affect fungal mycelium) all freshly isolated C. nicotianae protoplasts are killed. Again, by four hours, protoplasts start to regain their resistance to cercosporin with approximately 50% of them surviving exposure to 10 μM cercosporin. The resumption of resistance to cercosporin is not due to the complete regeneration of the cell wall, for the protoplasts do not regain osmotic resistance until eight hours after isolation. We are currently working with fluorescent brighteners and fluorescein-tagged lectins to try and determine the components on the regenerating cell wall which are responsible for resistance.

We are also working to isolate cercosporin-sensitive mutants of C. nicotianae in order to determine definitively the mechanisms required for resistance to cercosporin. It is hoped that this information will be useful in developing new and better ways of controlling diseases caused by Cercospora species, and perhaps other plant pathogenic fungi as well. If we are in fact unable to induce resistance to the toxin in the host plant, perhaps we can approach the problem from an opposite, but equally effective angle, that is, trying to disrupt the resistance mechanisms of the fungus itself. This could be accomplished by the use of new, non-toxic chemical control which is targeted at disrupting a resistance mechanism rather than killing the fungus. Alternatively, it may be possible to selectively breed for compounds in the host which disrupt the fungal defense mechanisms. For example, tobacco plants which are only attacked by Cercospora nicotianae after the leaves begin to senesce, contain high concentrations of the fungal carotenoid inhibitor β-ionone while the plant is young, but contain significantly lower concentrations as the plant matures (D. Danehower, personal communication). Whether these changing levels of β-ionone have anything at all to do with the increased susceptibility of mature tobacco plants remains to be determined, but the possibility that plants can be genetically modified, not to resist pathogens, but to disrupt their virulence mechanisms is an important area that needs to be pursued.

II. Graduate Student:

Kimberly D. Gwinn, PhD Candidate in Plant Pathology. Thesis Topic: "Resistance to the Photosensitizing Fungal Toxin Cercosporin".

III. Postdoctoral Fellow:

Dr. Anne E. Jenns. Research Area: Protoplast Fusion in Tobacco.

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IV. Publications:

Daub, M. E. 1984. A cell culture approach to the development of disease resistance: studies on the phytotoxin cercosporin. Hort Sci. 19:382-387.

Hughes, K., D. Negrotto, M. Daub, and R. Meeusen. 1984. Free-radical stress response in paraquat-sensitive and resistant tobacco plants. Env. Exp. Bot. 24:151-157.

IX. Acknowledgements:

The excellent technical assistance of Mr. D. Wayne Pollard is greatly appreciated. This work has been supported by grants from R. J. Reynolds Corporation, The North Carolina Tobacco Foundation, The North Carolina Biotechnology Center, and the USDA Competitive Grants Program.

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PLANT PATHOLOGY

STATE 3572: EPIDEMIOLOGY AND CONTROL OF VIRUS DISEASES OF SOLANACEOUS CROPS

G. V. Gooding, Jr., Project Leader

I. Summary of Research

A breeding program was continued in cooperation with Drs. Wernsman, Chaplin and Sisson to incorporate resistance to potato virus Y from Havana 307 into the cultivars McNair 944 and NC 82. Cooperation was also continued with Drs. Rufty and Wernsman to incorporate resistance to Peronospora hyoscyami f. sp. tabacina, tobacco etch virus and tobacco vein mottling virus into burley tobacco cultivars.

Cross protection is being evaluated as a control measure for aphid transmitted viruses on tobacco in cooperation with plant pathologists at Catholic University in Chili. A severe strain of potato virus Y, similar to one that has occurred in North Carolina is endemic in some tobacco production-areas of Chile and provides an opportunity to evaluate cross protection as a control procedure. Before cross protection can be evaluated, data must be generated as follows:

1. A mild strain must be selected or induced that will protect against the necrotic strain(s).

2. The relative population of the severe strains must be determined if more than one occurs.

3. The effect of the mild strain on the yield and quality of the tobacco cultivars and other hosts produced in the areas of intended use of cross protection must be established.

4. The yield loss from the severe strain(s) on individual farms must be determined. This will identify farms where the concept can be tested. It, of course, would not be prudent to inoculate a field with the mild strain if it causes more loss on a given farm than a low incidence of the severe strain(s).

5. Adverse synergistic effects between the mild strain and other viruses in the area must not outweigh benefits gained.

All five of these preliminary steps have now been completed and the effectiveness of cross protection will be evaluated on the 1985-86 crop using on-farm tests.

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An assessment was made on the distribution and incidence of viruses in burley tobacco to aid in setting priorities for resistance development (Table 1).

Table 1. Distribution and incidence of viruses in burley tobacco in North Carolinain 1984.

		Virus incidence (%)[a]									
		TVMV[b]		TEV		AMV		TRSV		TSV	
Area	County	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	Graham	3.2-63.0	21.1	0-67.2	22.1	0-0.5	0.1	0-0.2	<0.1	0	0
2	Buncombe	9.8-84.7	39.3	0-71.6	11.7	0-0.1	0.1	0	0	0	0
2	Madison	0.4-66.4	22.2	0-7.5	0.9	0-1.3	0.2	0-1.5	0.3	0-2.0	0.3
2	Yancey	0.2-54.4	14.1	0-3.5	0.3	0-0.2	0.1	0-0.1	0.1	0	0
3	Watauga	2.0-54.4	31.8	0-3.8	0.7	0-1.4	0.3	0-0.3	0.1	0	0
	Mean		25.7		7.1		0.2		0.1		<0.1

[a]Data obtained from ten randomly selected fields in each county except Yancey where 20 fields were used. Average time of observations was at the early flowering stage of plant development.

[b]TVMV = tobacco vein mottling virus; TEV = tobacco etch virus: AMV = alfalfa mosaic virus; TRSV = tobacco ringspot virus; TSV = tobacco streak virus.

An intensive study of tobacco mosaic virus was made on a "problem" farm in Duplin County. Results were 1) primary infection from the soil occurred in fields not rotated and was spread to other fields; 2) resetting tobacco in the same spot from which mosaic-infected plants had been rogued resulted in infection from the soil of 3 of 86 reset plants; 3) dipping transplants in a 2% solution of dried mild or 1% crude papain reduced but did not eliminate infection from the soil; and 4) cleaning debris from and then spraying lawn mowers used for clipping transplants with 2.5% sodium hypochlorite (50% chlorox) inactivated tobacco mosaic virus.

II. Postdoctoral Fellows

Dr. E. K. Park. Research project is resistance to potato virus Y and control of aphid transmitted viruses by interference.

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III. Publications

Gooding, G. V., and Kennedy, G. G. Resistance in tobacco breeding line NC 744 to potato virus Y and inoculation by aphids. Plant Disease (in press).

Gooding, G. V., Jr., Wernsman, E. A., and Rufty, R. C. Reaction of Nicotiana tabacum L. cultivar Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus and Peronospora tabacina. Tobacco Science (in press).

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Evaluation of tobacco haploids for resistance to TMV, PVY and Meloidogyne incognita using detached leaves. (Abstr.) Phytopathology (in press).

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Association between resistance to potato virus Y and susceptibility to Peronospora tabacina in tobacco. (Abstr.). Crop Science (in press).

IV. Manuscripts in Review

Gooding, G. V., Jr. Relationship between strains of potato virus Y and breeding for resistance, cross protection and interference. Tobacco Science.

Gooding, G. V., Jr. Tobacco mosaic virus. in The Rod-Shaped Viruses. Van Regenmortel and Frankel-Conrat, eds. Plenum Publishing Corp., New York.

V. Papers Presented

Tobacco Workers Conference (31st)

1. Epidemiology of tobacco mosaic (15 min)
2. Control of tobacco mosaic (15 min)
3. Epidemiology and control of potato virus Y, tobacco vein mottling virus and tobacco etch virus (90 min)

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Title: NC 03773 Soilborne Diseases of Tobacco

Project Leader: H. D. Shew

I. Summary of Research

Black Shank. Studies on the influence of soil water potential, soil texture, and soil type on reproduction and survival of the black shank pathogen, Phytophthora parasitica var. nicotianae, and development of the black shank disease were completed. Sporangia of the pathogen were produced over a wide range of soil matric potentials, with maximum production occurring in the range of water potentials optimum for plant growth. The highest matric potential at which sporangia were produced was determined by soil texture. As the percentage of large soil pores increased, so did the highest matric potential which allowed sporangium production. Sporangium production was greatly reduced in soil types that were suppressive to development of black shank. The nature of the disease suppression in these soil types appears to be a combination of microbial, physical, and chemical factors. Disease incidence also was determined by matric potential, with disease occurring only in soil at matric potentials which were favorable to release and dispersal of zoospores. Soil type affected disease incidence by an affect on pore size distribution which determined dispersal of zoospores.

The effect of solanaceous plant species other than tobacco on soil populations of the black shank pathogen was determined in greenhouse and microplot tests. Seedlings were inoculated with a zoospore suspension and then assayed to determine if P. parasitica var. nicotianae could infect and reproduce on the roots of pepper, petunia, eggplant, tomato, niteshade, horsenettle, and datura. The pathogen did not cause root rot on any of the plants but was recovered from the roots and reproduced on the roots. Microplot tests indicated that populations of the black shank pathogen were higher in the root zone of eggplant, horsenettle, and pepper than in the root zone of corn and niteshade and in fallow soil. The epidemiological significance of these other solanaceous plants on maintaining populations of the black shank pathogen is still under investigation.

Microplot tests were used to determine if stabilizing selection occurs in isolates of the black shank pathogen selected for reduced metalaxyl (Ridomil) sensitivity after continuous metalaxyl exposure in the field. Isolates of P. parasitica var. nicotianae collected from control (no metalaxyl) and metalaxyl treated plots responded similarly in in vitro tests, indicating that no stabilizing selection occurred. These and previous results support the hypothesis that under field conditions metalaxyl use results in an increase in the proportion of least sensitive isolates in the pathogen population without a loss in fitness for survival or pathogenicity, and does not induce mutations for resistance to the fungicide. Metalaxyl should thus provide control of black shank over the long term without occurrence of fungicide resistance.

Granville Wilt. A cooperative study with Drs. Echandi and Powell of the Department of Plant Pathology was conducted on the effect of fall fumigation

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with and without plastic mulch on control of Granville wilt. The combination of fall fumigation with plastic mulch gave excellent control of the disease. Disease incidence was reduced from 47% in the control to 0% with fumigation with Telone C-17 plus mulch in a high reistance cultivar, and from 54% in the control to 5% in the fumigation plus mulch in a moderate resistant cultivar. This test will be continued and expanded in 1985.

Rhizoctonia. During the summer of 1984 a new leaf spot disease of flue-cured tobacco was observed throughout the flue-cured tobacco producing area of North Carolina. The disease caused severe losses in several fields and minor losses in numerous fields. The symptoms of the disease are similar to brown spot except the new disease also causes a shot-hole type lesion. The disease is caused by the perfect stage of the soilborne pathogen Rhizoctonia solani. The pathogen does not appear to be the same strain of R. solani that causes sore-shin of tobacco. Research is in progress on environmental conditions required for production of the infective stage of the pathogen and subsequent disease development.

II. Graduate Students

J. R. Sidebottom, M.S., Plant Pathology. "Influence of Soil Type and Soil Matric Potential on Sporangium Production and Infection of Tobacco by Phytophthora parasitica var. nicotianae."

R. J. Pulliam, M.S., Plant Pathology. "Infection and Colonization of Susceptible and Resistant Flue Cured Tobacco by Phytophthora parasitica var. nicotianae."

IV. Publications

Shew, H. D. 1984. In vitro growth response of Phytophthora parasitica var. nicotianae isolates to metalaxyl. Plant Disease 68:764-766.

Powell, N. T. and Shew, H. D. 1984. Granville wilt and its control. N. C. Agr. Ext. Ser., N. C. St. Univ., Agr. Bull. No. AG-335, 8 pp.

V. Manuscripts Accepted for Publication

Shew, H. D. 1985. Response of Phytophthora parasitica var. nicotianae to metalaxyl exposure. Plant Disease 69: (In press).

Csinos, A. S., Fortnum, B. A., Gayed, S. K., Reilly, J. J., and Shew, H. D. 1985. Methods for evaluation of chemicals for control of soilborne pathogens on tobacco. In, Methods for Evaluating Plant Fungicides, Nematicides, and Bactericides. Amer. Phytopathol. Soc., St. Paul: (In press).

Csinos, A. S., Fortnum, B. A., Powell, N. T., Reilly, J. J., and Shew, H. D. 1985. Resistance of tobacco cultivars and breeding lines to Phytophthora parasitica var. nicotianae. Tob. Sci.: (In press).

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VI. Manuscripts in Review

Shew, H. D., and Main, C. E. 1985. Rhizoctonia leaf spot of flue cured tobacco in North Carolina. Plant Disease 69:(In review).

Sidebottom, J. R., and Shew, H. D. 1985. Interaction of soil type and soil matric potential on infection of tobacco by Phytophthora parasitica var. nicotianae. Phytopathology 75:(In review).

Sidebottom, J. R. and Shew, H. D. 1985. Effects of soil texture and matric potential on sporangium production by Phytophthora parasitica var. nicotianae. Phytopathology 75:(In review).

VII. Papers Presented at Professional Meetings

Echandi, E., Shew, H. D. and Powell, N. T. 1985. Control of Granville wilt with fall fumigation and plastic mulch. 31st Tobacco Workers Conference, Pinehurst, NC.

Shew, H. D. 1984. The effects of metalaxyl exposure on the sensitivity of Phytophthora parasitica var. nicotianae isolates to metalaxyl. Phytopathology 74:810.

Shew, H. D. 1985. Influence of solanaceous plant species on soil populations of Phytophthora parasitica var. nicotianae. 31st Tobacco Workers Conference, Pinehurst, NC.

Sidebottom, J. R. and Shew, H. D. 1984. The effects of soil matric potential and soil texture on sporangial formation by Phytophthora parasitica var. nicotianae. Phytopathology 74:814.

VIII. Graduate Student Theses Completed

Sidebottom, J. R. M.S. Title: Influence of Soil Type and Soil Matric Potential on Sporangium Production and Infection of Tobacco by Phytophthora parasitica var. nicotianae.

IX. Acknowledgments

I acknowledge the excellent technical assistance of D. T. Glover, S. Bhikhai, and C. Gray in the reported research.

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Title: NC 09326 Effects of Water Stress on Expression of Host Resistance to a Phytophthora sp.

Project Leader: H. D. Shew

I. Summary of Research

Research was begun on the effects of soil water potential and plant water stress on development of the tobacco black shank disease. Initial studies have concentrated on the infection and colonization of black shank susceptible vs resistant seedlings. At high initial inoculum densities no difference in infection efficiency was observed between susceptible and resistant cultivars. Differences in disease observed in the field may thus be due to differences in root colonization or resistance expressed in mature plants.

Studies have also begun on the suitability of flue cured cultivars that range in level of black shank resistance for reproduction by the black shank pathogen. Soil from the root zone of cultivars with none, low, moderate, or high black shank resistance was assayed to determine Phytophthora populations. Mean populations were 43, 30, 16, and 5 propagules/gram of soil for cultivars with none, low, moderate, and high resistance. Inoculum production on a per gram root tissue basis will be quantified to determine the ability of the black shank pathogen to utilize host substrate for production of reproductive and storage propagules.

Growth chamber studies were conducted to determine if flooding predisposes seedlings to infection by the black shank pathogens. No differences in percentage infection of seedlings was observed with a flooding treatment.

IX. I acknowledge the assistance of D. T. Glover, C. Gray, and S. Bhikhai in this study.

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STUDIES ON PLANT DISEASE CONTROL, TOBACCO DISEASES

Harvey W. Spurr, Jr.

Professor of Plant Pathology and Research Leader,
Tobacco Pest and Biocontrol Research Unit, Agricultural Research
Service, USDA in cooperation with N. C. State University
State 05468

I. Summary of Research:

This project is concerned with disease control mechanisms and their application to achieve plant disease control in a practical and economical manner. The emphasis is on new approaches and new techniques for understanding and efficiently controlling diseases. Specific objectives of immediate importance are: determine how to use leaf microflora to control foliar disease, determine how to induce host resistance to disease and develop practical applications, determine how nematodes survive during periods of stress, dormancy and anhydrobiosis.

Research during 1984 was highlighted by the results described in the following paragraphs.

Tobacco blue mold: Soil drenches of metalaxyl applied to potted tobacco plants did not alter the germination of sporangia and subsequent penetration of leaf epidermal cells by Peronospora tabacina. Development of the pathogen in plants treated with fungicide before inoculation was limited to infection structures within epidermal cells; no intercellular hyphae or haustoria were formed. When metalaxyl was applied by soil drenches to blue mold-infected plants, within 48 hr, sporulation by P. tabacina was suppressed 96%, relative to the controls. Sporangiophores that developed on treated plants were incompletely formed and some sporangia showed abnormal morphology. Ultrastructural changes in the fungus were evident 24 hr after treatment; intercellular hyphae and haustoria were vacuolated and nuclei were condensed. Forty-eight hours after treatment, 52% of the haustoria and 77% of the intercellular hyphae were either necrotic or extensively vacuolated. Ninety-four percent of the haustoria from infected, untreated plants were encased in a single layer or amorphous, moderately electron-dense appositional material and 5% were encased in two layers of appositional material. The inner layer of encasement material was composed of amorphous material, whereas the outer layer consisted of both host wall-like and membranelike portions that stained more densely than the inner layer. (Cooperators: Gerald Van Dyke, Robert Trigiano)

Biological control of plant pathogens: A microcomputer simulation model was developed to predict the seasonal progression of Cercospora leafspot of peanuts; it was derived in part from an advisory model used for fungicide scheduling and in part from other published and unpublished data. Infection rate (new lesions per sporulating lesion per day) is a

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function of temperature, hours of relative humidity >95%, available uninfected tissue, and control agent residues. Latent and infectious periods are modeled as distributed delays. A submodel determines plant growth as a function of time, with a feedback function relating growth rate to disease. Predictions of disease progression closely fit field observations in validation tests. The model is being used to explore system sensitivity to weather, latent and infectious periods, fungicide, or biocontrol agent characteristics, and other variables. (Cooperator: Jack Bailey)

III. Postdoctoral Fellows:

Dr. Guy R. Knudsen, ARS-USDA Research Associate, Biological Control of Foliar Pathogens.

IV. Publications:

Trigiano, R. N., C. G. Van Dyke, H. W. Spurr, Jr. and D. J. Gray. 1984. Infection and colonization of tobacco callus by Peronospora tabacina. Phytopathology 74:280-285.

Bailey, J. E. and H. W. Spurr, Jr. 1984. Evaluation of two biological control organisms for early leafspot control of peanut, 1982. Fungicide and Nematicide Tests 39:132.

Spurr, H. W., Jr., G. V. Gooding and F. A. Todd. 1984. An investigation of metalaxyl soil treatments for tobacco blue mold control on the incidence and development of TMV and Granville wilt. Tob. Sci. 28:64-66.

Lam, J. J., Jr. and H. W. Spurr, Jr. 1984. Comparison of microbial leaf processing (MLP) to homogenized leaf curing (HLC). (Abstr.). Proc. 38th Ann. Tobacco Chemists' Research Conf. p. 29.

Trigiano, R. N., C. G. Van Dyke and H. W. Spurr, Jr. 1984. Effects of metalaxyl on Peronospora tabacina infecting tobacco. Phytopathology 74:1034-1040.

V. Manuscripts Accepted for Publication:

Spurr, H. W., Jr. 1985. Bioassays - a critical to biocontrol of plant disease. J. Agric. Entomol. 2: (In press).

Spurr, H. W., Jr. 1985. Mode of action of nematicides. Pages___ in: J. N. Sasser ed., An Advanced Treatise on Meloidogyne. (In press).

Spurr, H. W., Jr. and G. R. Knudsen. 1985. Biological control of leaf diseases with bacteria. Pages ____ in: C. E. Windels and S. Lindow eds., Biological Control Strategies in the Phylloplane. APS Books, St. Paul, MN. (In press).

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VI. Manuscripts in Review:

Knudsen, G. R., H. W. Spurr, Jr. and J. E. Bailey. 1985. A predictive simulation model for peanut leafspot. (Abstr.). Phytopathology 75:

VII. Papers Presented at Professional Meetings:

Knudsen, G. R. and H. W. Spurr, Jr. 1985. Microbial interactions which alter tobacco brown spot (Presentation: 31st Tobacco Workers Conference, Jan. 7-10, 1985, Pinehurst, NC.).

IX. Acknowledgements:

I thank Catherine Currin, Linda D. Daniel, and Henry "Buddy" Quick for technical assistance.

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NC 03791 EPIDEMIOLOGY AND CONTROL OF FOLIAR DISEASES OF TOBACCO
Project Leader: C. E.Main

I. Summary of Research:

Blue mold of tobacco is a foliar disease that is spread over long distances by tiny, airborne spores during the spring and early summer of the growing season. The pathogen, Peronospora tabacina Adam, is an obligate, fungal parasite which survives and reproduces only on tobacco and Nicotiana spp. Under favorable weather conditions the resulting epidemics can be "explosive" resulting in major crop loss. Although losses have decreased due to current control strategies, this disease remains a continuing threat to North Carolina and tobacco producing areas of the southeast.

Objectives

1. Monitor occurrence, distribution and importance of foliar diseases of tobacco in North Carolina.
2. Determine the source(s) of primary inoculum of Peronospora tabacina.
3. Develop improved control tactics for foliar diseases using fungicides, resistant cultivars and cultural practices.
4. Monitor the presence of ecotypes, strains and races of foliar pathogens.
5. Relate development and spread of foliar diseases to weather and climate.
6. Assess the impact of foliar disease on yield and value.
7. Establish cooperative efforts on foliar diseases with state, federal and international research centers.

Research is underway in breeding and genetics, biology of the pathogen and epidemiology of the disease, biometeorology of inoculum transport, fungicide testing and residue analyses. Several tobacco scientists at North Carolina State University are contributing to the understanding and control of this important disease problem. Formulation of disease control strategies is primarily the responsibility of the two extension pathology specialists assigned to tobacco, however, direct and frequent input by research pathologists and other interested parties is available.

In 1983 tobacco pathologists at NCSU, in cooperation with scientists from the University of Kentucky, proposed a comprehensive, 5-year plan of research on this important disease. The objectives were long-range and included extensive investigations on the disease, the pathogen population

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and the development of improved control strategies. Since 1982 both universities have been conducting a cooperative winter blue mold nursery in Puerto Rico. Five types of experiments were conducted in 1984. In addition to the important field testing of genetic and plant breeding materials developed at NCSU, the winter nursery also was heavily committed to testing of fungicides and development of fungicide application technology. Both these activities had proven nearly impossible in North Carolina because of inconsistencies in occurrence of blue mold and risks to the industry from artificial inoculation within North Carolina. The winter blue mold nursery has proven to be a vital aspect of the total blue mold program. We would hope to continue this effort on a year-to-year basis as long as the need exists.

Three research areas that have received major attention are the breeding and genetics program under Rebeca Rufty and E. A. Wernsman, the epidemiology studies of C. E. Main and the fungicide strategy program of P. B. Shoemaker. Using modern approaches, the breeding program has accomplished in 30 months what might have taken 5-6 years of traditional breeding. The NCSU Phytotron was used to study variation in the pathogen population as it relates to temperature tolerance (compared with the wild type prior to 1979) in an attempt to explain the unusual, yearly field recurrence of blue mold for the past five years. It is important to know if and how blue mold has changed to become an endemic problem in the southeast; this might then require different approaches to disease control.

Additional biological studies have been conducted by Dr. Spurr at Oxford related to pathogen variation and mechanism-of-action of the fungicide metalaxyl. It may also be necessary to monitor blue mold outbreaks during the spring and summer of 1985. We must be ever alert to the possible occurrence of a metalaxyl resistant strain of P. tabacina; approximately 85 percent of the tobacco acreage in North Carolina depends upon protection provided by this single site-specific fungicide.

Biology of the Pathogen, Peronospora tabacina

H. W. Spurr, Jr. and C. E. Main

Blue mold is a classical downy mildew disease caused by the fungus Peronospora tabacina Adam. The pathogen is an obligate parasite that is very difficult to work with and maintain in storage. P. tabacina is quite variable due to the coenocytic nature of the thallus (no cell walls), the multinucleate sporangiospores (15-30 nuclei per spore), and its prolific potential for sporulation.

Several isolates of P. tabacina have been collected and/or obtained from a number of locations since the epidemic of 1979. These locations include fields in North Carolina and Kentucky as well as from infections on wild Nicotiana repanda plants growing in SW Texas. An isolate has been obtained from Beltsville, Maryland that was collected in 1964. This isolate has been revived from long-term storage and will hopefully serve as a reference to the "state" of the blue mold pathogen prior to 1979. Research to date has focused on characterizing these isolates with the aim

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to better understand the pathogen population. It is important to know if these isolates, albeit a small sample, represent subspecies, strains or even races of the pathogen. Differences exist in symptom induction, virulence, germ tube characteristics and oospore morphology.

A new analytical isozyme procedure has recently been developed at Ft. Dietrick, Maryland and shows promise in differentiating species and subspecies in the downy mildew disease of maize. We are expanding our P. tabacina collection and have made plans to visit the Ft. Dietrick laboratory during 1985 to utilize this new technology in comparing our isolates. If isozyme differences are found, we will attempt to relate them to known genetic, pathogenic and morphological characters of the pathogen.

Epidemiology of the Blue Mold: The Epidemic per se

C. E. Main and M. A. Moss

Prior to 1979, daily maximum temperatures above 30° C during June and July were believed to be limiting for blue mold development in the field. Yet in 1979 and 1980 active blue mold occurred throughout the growing season. A shift to more temperature tolerant ecotypes of P. tabacina, within the pathogen population, may have played a role in the macroscale epidemics that have occurred each subsequent year. Computer mapping of 1980 maximum daily temperature data from a ten state tobacco region in the SE, together with production data obtained from the ASCS, was used to estimate postfacto the biogeographical distribution and potential impact (yield loss) of the wild type ecotype (15-30°C) vs temperature tolerant ecotypes (≥ 30°C) within the pathogen population. Predicted vs actual reported crop losses were compared graphically and statistically for 1980. This evidence, albeit indirect, indicated that only the presence of a temperature tolerant population could have caused the magnitude and distribution of crop loss reported by extension and research personnel within the ten state region.

Table 1 shows the results of Phytotron studies in which the direct effect of temperature on four selected isolates of P. tabacina was determined. Sporangiospores of all the isolates tested were capable of germination and infected the tobacco host giving rise to a new crop of spores and thus completing the disease cycle at daytime temperatures up to 36°C. Blue mold is no longer just a plant bed disease. Blue mold must now be considered a summertime, field disease that can occur (and has since 1979) each year in North Carolina. Disease control strategies must be developed to deal with this now recurring problem.

Current studies are concentrating on an understanding of the nature and importance of "systemic" blue mold. Under certain circumstances, as yet poorly understood, the fungal pathogen enters the vascular system of the tobacco plant, distorts the leaves and stunts the plant by disrupting normal meristematic activity. Entire tobacco fields can be affected and/or lost due to this phase of the disease. This was the primary cause of the $38 million loss in North Carolina burley tobacco in 1979. We are studying the etiology of this phenomenon to determine if "systemic" blue mold is

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Table 1: Effect of temperature on the sporulation of lesions and germination of the resulting sporangiospores of Peronospora tabacina.

Temperature (°C)	Sporulation				Germination (%)[a]			
	% Lesions		Spores/cm^2		Optimum Temp.		Production Temp.	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
34/23	87 [b]	85 a	10470 a	14422 a	70 a	75 a	46 a	61 a
36/25	65 b	64 b	1543 b	1437 b	36 b	48 b	15 b	32 b
38/27	25 c	0.4 c	17 c	0 c	0	-	0	-
40/29	0 d	0 d	0 d	0 c	-	-	-	-

[a] Germination tested under optimum temperature conditions and at temperature at which sporangia were produced.

[b] Means across four isolates; means with same letter (within columns) not significantly different at p=.05.

caused by a unique biotype of P. tabacina within the the pathogen population, or whether the symptoms result from some unique combination of host phenology, inoculum density, infection site, and environment.

Blue Mold Biometeorology Research
C. E. Main and J. M. Davis

For the last four years we have been working on the meteorological aspects of the occurrence of blue mold in the eastern U. S. Our work has centered around 1980 because of the blue mold disease reporting network that was finally in place during that year, and because of the limited (compared to succeeding years) use of chemical fungicides which might affect disease distribution patterns. The objectives were to use the 1980 disease and weather data to: (1) explain the observed spatial and temporal relationships between disease occurrence and temperature and precipitation, and (2) ascertain the source region for the inoculum using computer-assisted atmospheric trajectory analysis. Most efforts were directed at disease occurrence within North Carolina.

In the 1980 field epidemic, the lack of moisture on the tobacco leaves

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for spore germination appeared to be the main limiting factor for disease development. The disease continued to spread even when temperatures were outside the favorable range, provided moisture was available. Meteorological trajectory analysis indicated that there were many days in April, May and June 1980 when conditions were favorable for spore transport to North Carolina from the infected fields located to the south. Taking into account epidemiological latent periods, certain of these trajectories' dates were selected as representing the most probably periods of spore transport.

Recently we began using trajectory analysis to also identify inoculum source regions for blue mold occurrences in west-central Kentucky in 1981 and 1982. Extension pathologists had hypothesized that spores had been transported into the region from areas to the south and southwest. The results of our trajectory analysis work supports this hypothesis; however, before any definitive statements can be made, we must learn more about the distribution of tobacco in central and SW United States and the susceptibility of the varieties grown in these locations to blue mold.

Future work on the biometeorology of blue mold will take two courses: (1) a newly developed trajectory analysis model has become available which more adequately handles transport during the transitions in the planetary boundary layer at the day/night and night/day interfaces will be tested and used to model spore transport in future research programs. Preliminary work indicates that the model will more precisely define both spore source regions and the areas to which the spores are transported from a source region, and (2) work is also beginning at NCSU on a whole new series of atmospheric models designed to look at short-range (less than 10 km) atmospheric transport processes within and above canopies. This work is still in its infancy, but holds great promise for modeling the short-range transport of plant pathogens.

The Blue Mold Situation in Mexico and Texas

Documented evidence of P. tabacina resistance to metalaxyl has been reported from the gulf coast tobacco production area of Mexico around Vera Cruz. Reports from TABCOS MEXICANOS as of January, 1985 indicate that all the native, cigar type tobacco was rather severely infected with blue mold that does not respond to applications of Ridomil . Tobacco in the Tepic production area of the NW coast is infected to a lesser degree and shows no signs of metalaxyl resistance at this time. Continued surveilence and monitoring of blue mold infections on Nicotiana repanda growing wild in SW Texas will be necessary to determine if and when the metalaxyl resistant strain of P. tabacina may move into the United States via that route. This avenue of entry, using Texas as a bridge in space, will require careful scrutiny. We hope to cooperate closely with plant pathologists from the University of Kentucky on this monitoring aspect of the project. Long distance inoculum studies have been initiated in cooperation with the pathologists in Kentucky. It may be necessary to arrange a first hand, fact finding visit to the tobacco areas of Mexico to fully understand the

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nature, scope and implications of important inoculum source.

The Blue Mold Breeding Program

Rebeca C. Rufty, E. A. Wernsman and C. E. Main

The primary objective of the blue mold breeding program is to develop blue mold resistant flue-cured and burley genotypes of acceptable agronomic quality which may be used either as pure lines or in hybrid form for cultivar use. Initially, hybridizations were made between putative resistant genotypes Z-3 and Ovens 62 from Europe and Australia and susceptible U. S. cultivars McNair 944, NC 95, Ky 15, KY 17, and NC 13. F_1 hybrids from these crosses were subjected to the anther culture procedure generating approximately 400 dihaploid (DH) lines which were evaluated for blue mold resistance in Gurabo, Puerto Rico in 1983-84. Significant differences in blue mold reaction were found among DH's, and approximately forty of these were selected for further testing. Selected DH's were planted in Laurel Springs, North Carolina. However, disease reaction under North Carolina conditions could not be determined because blue mold did not develop in 1984. A similar test was conducted in Gurabo, Puerto Rico in the winter of 1984-85 which included the selected DH's as well as several European burley cultivars believed to be blue mold resistant. Eighteen DH's were selected from this winter nursery on the basis of apparent resistance and superior agronomic traits. All European burley cultivars were found to be susceptible.

The Puerto Rico winter nursery has enabled us to get information on breeding lines that could not have been obtained in North Carolina due to the absence of blue mold in the state for the last two years. Highly susceptible DH's have been discarded based on data collected in Puerto Rico and further testing will be done with only a few promising genotypes. Without this facility, the breeding program would have been delayed considerably since progress depends on the breeder's ability to make selections under disease pressure. Environmental conditions in Puerto Rico are conducive to blue mold development, thus ensuring that breeding materials can be evaluated in at least one location per year. Should blue mold develop in North Carolina in future years, then information could be obtained from both locations thereby accelerating the overall progress of the program.

Resistance to Peronospora tabacina has not been observed in the germplasm collection of tobacco, Nicotiana tabacum. Programs to induce mutations conferring disease resistance or transfers of genetic mechanisms for disease resistance from alien Nicotiana species to tobacco have resulted in some successes. In interspecific transfers of blue mold resistance to tobacco, genes of interest in the alien Nicotiana species must exhibit dominance or partial dominance, and the character must be qualtatively inherited. Three flue-cured cultivars with some resistance to P. tabacina (Ovens 62, Sirone and Sirogo) were obtained from the Australian blue mold breeding program. The genetic mechanism for disease resistance in these cultivars should exhibit some degree of dominance.

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In North Carolina environments, Ovens 62, Sirone, and Sirogo exhibit poor plant form, produce abundant suckers, and appear to be low yielding. Nevertheless, Ovens 62 and the F_1 hybrid of Ovens 62 with susceptible American cultivar, McNair 944, have exhibited some resistance to this disease in the blue mold nursery at Gurabo, Puerto Rico. Any commercial utilization of such hybrids would require cytoplasmic male-sterile females as parents for efficient hybrid seed production. One objective of the 1984 breeding program was to evaluate the agronomic performance and leaf chemical constituents of normal and cytoplasmic male-sterile (cms) F_1 hybrids of the Australian cultivars with popular American varieties.

During 1984 Dr. Wernsman converted four American flue-cured cultivars, McNair 944, Coker 347, Speight G-28, and Speight G-70, to cms parents. The N. suaveolens source of cms was utilized, since 1983 results at Gurabo, Puerto Rico, indicated that this cytoplasm had no effect on the blue mold disease reaction. Each Australian cultivar, Ovens 62, Sirone, and Sirogo, was crossed to the normal (fertile) and cms (sterile) form of each American cultivar. The three Australian cultivars, the four American cultivars in fertile and cms condition, and the sterile and fertile F_1 hybrids of each Australian cultivar crossed with each American cultivar were grown and evaluated in 1984 at Whiteville and Kinston, North Carolina. These 35 genotypes were grown in three replications of a randomized complete block design with 20 competitive plants per plot at the two locations. Entries were evaluated for leaf yields, leaf quality as measured by grade index, and percentages of total alkaloids as nicotine and reducing sugars in the cured leaf. Cured leaf of Ovens 62, cms Speight G-70 x Ovens 62, cms Speight G-70, cms McNair 944 x Ovens 62 and cms McNair 944 were submitted to R. J. Reynolds Tobacco Company for physical evaluation, cigaret manufacture, and smoke panel evaluation.

Male-sterile and male-fertile cultivars and hybrids did not differ from each other for any of the parameters measured. This suggests that the N. suaveolens cytoplasm had no differential effect on cultivar or hybrid performance, and that the genotypes of the American cultivars had been adequately recovered in their conversion to cms. The Australian cultivars, as expected, were significantly lower yielding than American cultivars and the F_1 hybrids. However, their cured leaf quality was equal or superior to the American varieties. Furthermore, they possessed a more "balanced" leaf chemistry in terms of total alkaloids as nicotine, reducing sugars, and sugar-nicotine ratios. F_1 hybrids were intermediate between the parents in agronomic performance and chemical composition, and were superior to the American cultivars in physical aspects of leaf quality. Results of smoke panel evaluations are unavailable at this time.

Although these results are preliminary, they suggest that F_1 hybrids of American cultivars with Ovens 62 could be considered as an "emergency" measure for blue mold control. If a Ridomil®-resistant strain of P. tabacina were to arise, these lines could be increased and, together with the use of protectant fungicides, could help avoid a potentially crisis situation. In particular, the F_1 hybrid of McNair 944 x Ovens 62 was intermediate in plant form, produced 3149 kg/ha cured leaf, had a grade index of 50, 2.21% total alkaloids and 18.8% reducing sugars. This

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performance was competitive with that of the American cultivars in this test.

Protectant Fungicides and Alternative Strategies for Blue Mold Control

P. B. Shoemaker and C. E. Main

Strategies for blue mold control were evaluated in four replicated research tests, including four in North Carolina (Clayton, Waynesville, Burnsville and Laurel Springs) and a fifth at Gurabo, Puerto Rico in 1984. The primary objective was to evaluate two materials, mancozeb and Galben® (benalaxyl) at various rates, timing schedules and application methods as alternatives to the almost sole use of Ridomil®(metalaxyl) for blue mold control. Approximately 85% of the tobacco acreage in North Carolina is treated with a pre-season soil application of Ridomil®. The documented presence of pathogen resistance to this site-specific fungicide in Central America and Mexico makes it imperative that alternative materials and strategies be developed and contingency plans be formalized.

A second objective was to obtain chemical residue data on the cured leaf from the different strategy treatments. Blue mold did not develop at any of the N. C. test sites in 1984, thus it was impossible to evaluate the efficacy of the disease control treatments. However, residue samples from experimental plots sprayed with various rates of mancozeb and at differing timing schedules were collected and are presently being analyzed. Preliminary data indicate that residue levels of ethylene bis dithiocarbamate (EBDC) and ethylenethiourea (ETU) are within acceptable ranges for both domestic and foreign markets (re: next section).

A severe, natural epidemic of blue mold did occur in the Puerto Rico winter nursery tests providing important data on the efficacy of protectant fungicides for controlling blue mold. Under the tropical conditions of the test, a 5-day spray schedule with the protectant fungicide mancozeb provided significantly better control than either a 7 or 10-day schedule. Galben® plus mancozeb at 14-day intervals provided excellent early season control compared to an unsprayed check, but resulted in surprisingly high disease ratings at mid-and-late season readings. Galben®is being considered for labeling on tobacco for blue mold and further testing will be necessary to fully optimize its use strategy. Results from the 1984 tests, in conjunction with data from 1982 and 1983 tests, support the NCSU contingency plan, i.e. to recommend an alternative strategy control program for blue mold control should resistance to Ridomil® become a reality in North Carolina.

Plans are to continue evaluating alternative fungicide strategies for blue mold and to collect residue samples for further analyses. It is also proposed to monitor any natural occurrences of blue mold in North Carolina during 1985 to detect as early as possible Ridomil resistant strains of P. tabacina, should they occur. This could provide sufficient lead time for initiating our contingency plan and recommending alternative fungicides to growers. Together with the blue mold resistant cultivars being developed

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in the breeding program, these strategies could avoid a potentially catastropic situation for the tobacco growers of North Carolina.

Fungicide Residue Studies
T. J. Sheets, P. B. Shoemaker and C. E. Main

An analytical method was developed to determine ethylene bis dithiocarbamate (EBDC) and ethylenethiourea (ETU) residues on flue-cured and burley tobacco treated for control of blue mold. The method used for EDBC was an electron capture gas-chromatography technique, and that for ETU was an HPLC with UV detection.

Samples of burley from 1983 blue mold field experiments at Laurel Springs and Waynesville were analyzed for both residue components. When mancozeb was applied at 5-day intervals, EBDC residues averaged 54, 88, and 103 ppm and ETU residues averaged 0.13, 0.16, and 0.17 ppm for cured leaf from lower, middle, and upper stalk positions, respectively. With 14-day intervals, residues were much lower (<0.05 to 25 ppm). Burley samples from 1984 are presently being analyzed.

A field residue study was also conducted on flue-cured tobacco at Clayton in 1984. When mancozeb (2 lb ai/100 gal) was applied every 7 days from late May to early July, residues of EBDC for the four harvests were 23.0, 14.2, 9.5, and <0.05 ppm. With a 14-day interval, residues were lower. ETU residues were detected only in fourth harvest samples at levels of 0.07 to 0.10 ppm.

Plans are to continue these type of studies in connection with fungicide strategy experiments in 1985.

II. Graduate Students: Michael A. Moss (1984 recipient of the E. G. Moss scholarship for outstanding thesis research on tobacco).

Epidemiology of the blue mold disease of tobacco caused by Peronospora tabacina.

III. Postdoctoral Fellows: None

IV. Publications:

Campbell, C. L., W. R. Jacobi, N. T. Powell, and C. E. Main. 1984. Analysis of disease progression and the randomness of occurrence of infected plants during tobacco black shank epidemics. Phytopathology 74:230-235.

Davis, J. M., and C. E. Main. 1984. A regional analysis of the meteorological aspects of the spread and development of blue mold of tobacco. Boundry-Layer Meteorology 28:271-304.

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V. Manuscripts Accepted for Publication:

Main, C. E., J. M. Davis, and M. A. Moss. The biometeorology of blue mold of tobacco. Part I. A case study in the epidemiology of the disease and in the movement and dispersal of inoculum. In McKenzie, et. al. [Eds.] The Movement and Dispersal of Biotic Agents in Agriculture.

Davis, J. M., C. E. Main, and W. C. Nesmith. The biometeorology of blue mold of tobacco. Part II. The evidence for long range sporangiospore transport. In MacKenzie, D. R., C. Barfield, R. D. Berger, and G. G. Kennedy [Eds.]. The Movement and Dispersal of Biotic Agents in Agriculture. University Graphics, North Carolina State University, Raleigh, NC.

VI. Manuscripts in Review:

Kirby, H. W., and C. E. Main. Identification and analysis of factors related to acceptance of integrated pest management among tobacco farmers. Plant Disease.

Moss, M. A., and C. E. Main. Evidence of temperature tolerance in Peronospora tabacina. Phytopathology.

Shew, H. D., and C. E. Main. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina. Phytopathology.

VII. Papers Presented at Professional Meetings:

Davis, J. M., and C. E. Main. 1984. Meteorological aspects of the spread and development of blue mold on tobacco in North Carolina. APS/CPS Annual Meeting, Guelph, Ontario, Canada. August 12-16, 1984.

Main, C. E. 1984. Summary of the blue mold research project in North Carolina, USA. 8th International Tobacco Scientific Congress, Vienna, Austria. October 7-12, 1984.

Main, C. E., and J. M. Davis. 1984. A case-history: Tobacco blue mold. International Conference on the Movement and Dispersal of Biotic Agents. Louisiana State University, Baton Rouge, La. October 17-19, 1984.

Main, C. E. 1985. A report on blue mold research in Europe and the 8th International Tobacco Scientific Congress, Vienna, Austria. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

Main, C. E., and J. M. Davis. 1985. Analysis of long-distance sporangiospore transport of Peronospora tabacina. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

Moss, M. A., and C. E. Main. 1985. Evidence for temperature tolerance in Peronospora tabacina. 31st Tobacco Workers' Conference,

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Pinehurst, NC. January 7-10, 1985.

Shoemaker, P. B., and C. E. Main. 1985. Fungicides and strategies for blue mold control. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

Leidy, R. B., T. J. Sheets, P. B. Shoemaker, C. E. Main, and N. T. Powell. 1985. Residues of EBDC and ETV in tobacco after application of mancozeb. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

Main, C. E. 1985. A report on the Puerto Rico blue mold tobacco nursery - a valuable research facility. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

Johnson, C. S., and C. E. Main. 1985. Crop loss assessment and variety trade-off models for tobacco mosaic virus. 31st Tobacco Workers' Conference, Pinehurst, NC. January 7-10, 1985.

VIII. Graduate Student Theses Completed During Reporting Period:

None

IX. Acknowledgements:

The blue mold research program at North Carolina State University is broad and complex involving many scientists. It is a team effort. It is my privilege to serve as coordinator of the blue mold research program and intereact with scientists from other departments, states and agencies concerned with this important disease. Special thanks are given to J. M. Davis (Meteorology), J. Kuc' and William Nesmith (University of Kentucky), N. T. Powell and P. B. Shoemaker (extension tobacco pathology), Rebeca Rufty and Earl Wernsman (Crop Science), H. W. Spurr, Jr. (USDA, Oxford), and T. J. Sheets (Pesticide Residue Research Laboratory).

A special thanks is offered to Jerry Shiffert and Arnold Mitchem and to the R. J. Reynolds Tobacco Company for the support and encouragement provided to the blue mold research program in 1984.

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Title: NC05507 Disease Control Strategies for Burley Tobacco and Vegetables in Western North Carolina

Project Leader: P. B. Shoemaker

I. Summary of Research:

Alternative fungicides and strategies for blue mold. Alternative strategies for blue mold control were evaluated in four replicated research tests, including three in North Carolina (Waynesville, Burnsville and Laurel Springs) and a fourth at Gurabo, Puerto Rico in 1984. The primary objective was to evaluate two materials, mancozeb and Galben® (benalaxyl) at various rates, timing schedules and application methods as alternatives to Ridomil® (metalaxyl) for blue mold control. A second objective was to obtain chemical residue information on the different treatments. Blue mold did not develop at the N. C. test sites in 1984, thus it was not possible to evaluate treatments for disease control. However, samples from treatments sprayed with various rates and timing schedules with mancozeb were collected and will be analyzed by the Residue Research Laboratory, NCSU in Spring 1985. Preliminary data from residue analyses indicate that ethylene bis dithiocarbamate (EBDC) and ethylene thiourea (ETU) levels are within present acceptable ranges. A severe blue mold outbreak did occur in the Puerto Rico tests and has provided additional data on the use of protectant fungicides for this disease. Under the conditions of the test, sprays with the protectant mancozeb applied at 5-day intervals provided significantly better control than sprays at 7 or 10-day intervals. Galben® plus mancozeb at 14-day intervals provided excellent control at an early disease reading compared to an unsprayed check, but had more disease at a later reading. These results indicate that further testing with Galben® is necessary to optimize its use strategy. In summary, results from the 1984 tests in conjunction with those from 1982 and 1983 provide data to support an alternative control strategy recommendation for blue mold should resistance to Ridomil® become a reality. In 1985, we will continue to evaluate alternative fungicide strategies for blue mold and collect samples sprayed with different fungicides for residue analyses. We must be certain that alternative controls which could be recommended for growers use have residues which are deemed acceptable and safe. In addition, we propose to monitor any natural occurrences of blue mold for possible Ridomil® resistant strains of *P. tabacina* so as to be in the best position for recommending alternative strategies to growers.

Influence of plant and row spacing and protectant fungicide spray interval on blue mold epidemics. Three plant by row spacings (14 X 42, 18 X 48 and 22 X 54 inches) and four protectant fungicide spray intervals (0, 5, 7 and 10-day) were compared in a factorial experiment to determine their influence on blue mold development. Blue mold did not develop, so effect of plant and row spacing on yield and quality in the absence of blue mold was the only data available from this test. Close (14 X 42 inches) plant spacing resulted in significantly higher yields (27.4%) and value (24.6%) per acre than wide (22 X 54 inches) plant spacing. Fungicide treatments did not affect yield. In a 1983 test where blue mold was present, 5-day fungicide treatments increased yields 25.0% for close plant spacing and only 3.5% for wide plant spacing. Information from 3 years of testing is being prepared for publication.

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Fumigants for black root rot control on burley tobacco. Ten soil fumigation treatments were evaluated in a replicated test on a grower's farm with a known history of black root rot (Thielaviopsis basicola). Differences in growth were noted between treatments 1 month after transplanting, but by the time of harvest, growth appeared uniform. Soil assays in selected treatments at harvest indicated that root knot nematodes were absent, but T. basicola was recovered in the check (14 of 128 subsamples) and not in an 8 gal/A Vorlex treatment (0 of 128 subsamples). Differences in yield and value were not significant for any of the fumigants or fumigant by variety combinations. Va 509 (low resistance) performed slightly better than Ky 17 (high resistance), but not significantly. This and tests in recent years have failed to demonstrate an advantage to using a fumigant in burley tobacco in W. N. C. for black root rot control. Future studies will be directed at improving our understanding of the nature and scope of this soil-borne disease problem.

IV. Publications:

Shoemaker, P. B. 1984. Evaluation of systemic fungicides for tobacco blue mold, 1982. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 39:149.

Shoemaker, P. B. 1984. Evaluation of protectant fungicides for tobacco blue mold, 1982. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 39:149.

V. Manuscripts Accepted for Publication:

Shoemaker, P. B. 1985. Protectant fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath Soc. 20 (In-press).

Shoemaker, P. B. 1985. Foliar applied systemic fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 40 (In-press).

Shoeamker, P. B. 1985. Soil applied systemic fungicides for tobacco blue mold, 1983. Fungicide and Nematicide Tests, Am. Phytopath. Soc. 40 (In-press).

VII. Papers Presented at Professional Meetings:

Shoemaker, P. B. and C. E. Main. 1985. Fungicides and strategies for blue mold control. The 31st Tobacco Workers Conference, Pinehurst, NC, January 7-10, 1985.

IX. Acknowledgements: I wish to thank: Superintendents J. E. Edwards, D. F. Tugman, respectively, Mountain Research Station, Waynesville and Upper Mountain Research Station, Laurel Springs and their staffs for on-site assistance, especially Hugh Russell; various companies, including, Ciba-Geigy, Montedison USA, Rohm & Haas, NOR-AM Agricultural Chemical, Great Lakes Chemical and R. J. Reynolds for materials and financial assistance; and especially, M. H. Henry and D. C. Milks for their technical assistance and expertise.

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Title: NC 3901 Plant Bed and Field Management of Flue-Cured Tobacco

Project Leaders: G. S. Miner

I. Summary of Research:

Experiments were conducted in two areas of research in 1984. One area involved an assessment of the effects of N rates and topping practices on yield, quality index, and contents of reducing sugars and total alkaloids in two varieties of flue-cured tobacco. A major area of interest, the effects of these treatments on organic constituents of green, yellowed, and flue-cured tobacco is presently being evaluated in the laboratory. This work is being conducted jointly with Dr. W. W. Weeks.

The second area of research involved the continued assessment of the effects of subsoiling on yield, grade index, and chemical constituents of flue-cured tobacco. This work is being conducted in cooperation with a graduate student, David Hardy, and Dr. M. J. Vepraskas. Dr. Vepraskas has reported on the relationship between growth response and soil physical properties elsewhere in this annual report. This summary will concentrate on the effects of subsoiling on yield and grade index. The material is presently being analyzed for reducing sugars, total alkaloids, and inorganic nutrient content. The latter will be related to subsoiling effects and depth to clay.

A. N x MH Treatments x Variety: This experiment was located at the Oxford Tobacco Research Station and involved the application of 30% less, 15% less, and the recommended rate of N on cv. Speight G28 and Coker 347. Some sites had no N in lieu of the 30% less than recommended rate. Additionally, handsuckering versus MH application was imposed on each combination of variety and N rate. All treatments were replicated three times and arranged in split plots with combinations of N rates and suckering treatments in whole plots and varieties in sub plots. Green leaf samples were collected on the day of each priming, stemmed, and freeze dried. Leaf samples were collected at the end of the yellowing phase of curing from each priming and were processed as described for green samples. Composit cured leaf samples were collected from each plot after curing.

There were no significant differences in yield due to N rates or MH treatments. There were no significant interactions among treatments but there was a significant difference in yield between varieties with C347 averaging 2773 lb/A compared to 2657 lb/A for G28.

MH treatments and all treatment interactions gave no significant differences in grade indices. However, the grade index for G28 averaged 42.2 and was significantly higher than the 29.2 obtained for C347. Grade index was significantly increased by higher rates of N and averaged 34.4, 34.0, and 38.7 for the 40, 55, and 70 lb/A rates of N, respectively.

The concentrations of reducing sugars and total alkaloids were significantly affected by rates of N and significant variety effects

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occurred only for total alkaloids. Suckering treatments and all treatment interactions for concentrations of reducing sugars and total alkaloids were not significant. Reducing sugar decreased from 20.6% for the 40 lb/A rate of N to 17.7% for the 55 and 70 lb/A N rates. Total alkaloids increased from 2.38% to 2.93% and 3.04% for the 40, 55, and 70 lb/A N rates, respectively. Total alkaloids averages 2.31% and 3.25% for G28 and C347, respectively.

All green, yellow and cured leaf samples are presently being evaluated for organic constituent profiles to determine how they are affected by the treatments that were imposed and to determine the changes they undergo during the curing process.

B. Subsoiling by N rates: A total of 10 experiments were conducted covering a wide range of soil conditions. Only yield and grade index data are available at this time.

A summary of the main effects of tillage and N rates on yield is given in table 1. Subsoiling significantly increased yield in 5 of the 10 experiments. The relation of this response to soil physical properties is discussed by Dr. Vepraskas in his report. Only 4 experiments had significant yield responses to N. Two of these were at sites where no N was applied at the lowest rates.

There were no significant differences in grade index due to tillage at any location (Table 2). Increasing N rate decreased grade index at one location and increased it at a second location (Table 2). There were no significant differences in grade index among N rates at the remaining locations.

The effects of these treatments on nutrient recovery is presently being determined. This will assist us in determining if subsoiling benefits may be partially due to increased availability of various nutrients.

II. Graduate Students: One M.S. Candidate
One M.S. Candidate co-chaired with Dr. Raper
One Ph.D. Candidate co-chaired with Dr. Peedin

VI. Manuscripts in Review:

(1) Sene, M., M.J. Vepraskas, G.S. Miner and G. F. Peedin. Relationships among relative tobacco yield increase to subsoiling and soil physical properties. For Agron. J.

(2) Vepraskas, M.J., G. S. Miner, and G. F. Peedin. Effects of subsoiling, bulk density, and cone index on tobacco root growth. For Agron. J.

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Table 1. The main effects of tillage and rate of N application on flue-cured tobacco yield at 10 locations in 1984.

Location[b/]	Tillage		Statistical Significance	N Rates[a/]			Statistical Significance
	Not Subsoiled	Subsoiled		(0) or 1	2	3	
Nashville	1326	1782	**	1489	1614	1558	NS
Kinston	2694	2441	NS	2578	2626	2500	NS
Kinston	2259	2701	**	2428	2509	2504	NS
Kinston (RS)	2957	3105	NS	2897	2916	3280	*
Kinston (RS)	2528	2849	**	—	—	—	
Clayton (RS)	1879	1986	*	1780	1970	2047	**
Clayton (RS)	2134	2222	NS	(1506)	2454	2574	**
Rocky Mount (RS)	2614	2939	**	(2168)	3114	3047	**
Rocky Mount (RS)	2831	2856	NS	2685	2926	2919	NS
Whiteville (RS)	3389	3518	NS	3402	3298	3660	NS

a/ (0) = no N applied; 1, 2, and 3 = 30% less, 15% less, and the recommended rate of N respectively. Only one rate of N applied at location with —.

b/ (RS) = research station. Other locations on farmers fields.

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Table 2. The main effects of tillage and rates of N application on grade index of flue-cured tobacco at 10 locations in 1984.

Location[b/]	Tillage		Statistical Significance	N Rates[a/]			Statistical Significance
	Not Subsoiled	Subsoiled		(0) or 1	2	3	
Nashville	46.4	44.9	NS	43.4	46.6	47.0	NS
Kinston	60.8	62.5	NS	62.3	61.0	61.7	NS
Kinston	55.5	56.4	NS	56.5	57.0	56.3	NS
Kinston (RS)	48.4	47.0	NS	48.7	47.2	47.2	NS
Kinston (RS)	45.2	44.2	NS	—	—	—	
Clayton (RS)	55.4	54.8	NS	57.2	54.9	53.2	**
Clayton (RS)	56.0	58.4	NS	(56.2)	56.6	59.0	NS
Rocky Mount (RS)	53.3	51.8	NS	(51.7)	51.9	53.9	*
Rocky Mount (RS)	48.9	48.9	NS	49.2	49.9	47.7	NS
Whiteville (RS)	46.7	47.3	NS	44.8	49.3	47.0	NS

a/ (0) = no N applied; 1, 2, and 3 = 30% less, 15% less, and the recommended rate of N, respectively. Only one rate of N applied at location with —.

b/ (RS) = research station. Other locations on farmers field.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

Title: NC 03897 Effects of Selected Soil Physical Properties and Subsoiling on Root Growth and Crop Yield Response.

Project Leader: M. J. Vepraskas

I. Summary of Research:

This project has the following objectives:

A. Determine how selected soil physical properties affect yield response to subsoiling and root growth.

B. Develop means to predict yield response to subsoiling for specific field sites.

Ten fields were studied in 1984 where flue-cured tobacco yields of subsoiled and not-subsoiled treatments were measured, as were soil properties and root distributions. This study was initiated in 1981, and a total of 32 separate field locations have been examined to date, in cooperation with Drs. G. S. Miner (Soil Science) and G. F. Peedin (Crop Science). For all locations yield responses to subsoiling (YR's) ranged from -6 to 25%. Subsoiling significantly increased yields over the nonsubsoiled treatment where YR's were >10%. No soil type or location consistently had yields significantly increased by subsoiling each year. Soils responding to subsoiling had tillage pans in either the lower Ap horizon or just below it. These pans had bulk densities $\geq$ 1.65 Mg m^{-3} and reduced root penetration into the subsoil. Soils having bulk densities < 1.65 Mg m^{-3} generally did not respond to subsoiling. For soils with tillage pans, YR's were >10% during the dry years where cumulative rainfall for the months of June, July, and August was < 33 cm. Yield responses were generally <10% for higher rainfall levels if the rainfall was well distributed. Identification of soils responsive to subsoiling is best made from bulk density values of the lower Ap horizon or the next lower horizon. Where bulk densities are $\geq$ 1.65 Mg m^{-3} subsoiling should be used to disrupt the tillage pan.

IV. Publications:

Vepraskas, M. J. 1984. Cone index of loamy sands as influenced by pore size distribution and effective stress. Soil Sci. Soc. Am. J. 48:1220-1225.

V. Manuscripts Accepted for Publication:

Sene, M., M. J. Vepraskas, G. C. Naderman, and H. P. Denton. 1985. Relationships of soil texture and structure ot corn yield responses to subsoiling. Soil Sci. Soc. Am. J. 49:(In Press).

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

VI. Manuscripts in Review:

Vepraskas, M. J., and G. S. Miner. 1985. Effects of Subsoiling, Bulk Density, and Cone Index on Tobacco Root Growth. Soil Sci. Soc. Am. J. (In Review).

VII. Papers Presented at Professional Meetings:

Vepraskas, M. J., M. Sene, G. C. Naderman, and H. P. Denton. Relationships of Soil Texture and Structure to Corn Yield Responses to Subsoiling. Presented at the 1984 Annual Meeting of the Soil Sci. Soc. of Am., Las Vegas, NV.

IX. Acknowledgements:

I wish to thank Mark Carpenter and James Wade for excellent technical assistance.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

Title: NCARS 03847 Effects of Selected Soil Management Systems on Erosion, Water Conservation and Plant Growth Conditions

Project Leaders: D.K. Cassel, C.B. McCants, G.R. Bathke

I. Summary of Research:

The objective of the research is to develop and investigate tillage systems for tobacco production which will increase water use efficiency, reduce soil erosion and increase the yield and quality of cured leaves. A better understanding of the optimum soil physical environment for tobacco production is a prerequisite to successful efforts for improving on current cultural practices. Soil physical properties and plant growth conditions under soil management systems were studied at three locations: Central Crops Research Station, Clayton, NC; Oxford Tobacco Research Station, Oxford, NC; Upper Piedmont Research Station, Reidsville, NC. Tillage system combinations evaluated were: fall vs. spring primary tillage; ridge vs. level row configuration; cultivation vs. no cultivation vs. cover crop. Soil samples and field measurements were taken in the spring after transplanting and in the fall during the harvest period. Surface bulk density and mechanical impedance, a measure of soil compaction and soil strength were greater when taken from locations between the rows than in the rows. Soil density and strength were also greater in level than ridged rows, and in fall compared to spring tilled areas. Differences observed in bulk density and mechanical impedance were greater when measured in the spring than fall. Soil density and strength reached growth limiting conditions under level row configuration treatments. Transplanting problems due to high soil strength resulted in poor stands in the fall primary tillage treatments at the Oxford location. The disc-opener on the tobacco transplanter inadequately penetrated the soil surface thereby causing the seedlings to be planted too shallow. A suitable plant population was attained, but plant development lagged far behind other treatments. Tobacco yield and quality were reduced by the level treatments at all locations. Total season runoff and erosion were greater under spring tillage and greatest when cultivated. Initially, runoff events were of similar size over all treatments, but showed relative decreases on no-till vs. other plots. Further detailed analyses are being done at this time.

IIB. Special Students: Mr. Kirk McEachern - under the R.J. Reynolds Undergraduate Research Apprenticeship Program

VII. Papers Presented at Professional Meetings:

A Semi-Portable Multislot Divisor for Erosion and Runoff Measurements. G.R. Bathke
Presented at 76th Annual ASA-CSSA-SSSA Meetings, Las Vegas, NV, November 25-30, 1984.

IX. Acknowledgements:

We wish to thank F.G. Averette, H.P. Denton, R.W. Lemons, K. McEachern, and A.D. Worsham for excellent technical assistance.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

Title: NC 03659 Environmental Determinants of Crop Growth and Soil Productivity

Project Leader: C. D. Raper, Jr.

I. Summary of Research:

During the last year, we have continued to investigate the effects of root-zone environmental factors on the interrelationship between nitrogen uptake and carbon assimilation in tobacco plants. Experiments have concentrated on nitrogen uptake and utilization in tobacco plants as affected by (A) nitrogen source, (B) nitrogen concentration, and (C) nitrogen stress. Experiments also have been conducted to evaluate the effects of leaf water stress on photosynthetic activity and photosynthate partitioning.

A. Effects of Nitrogen Source

The effect of root-zone acidity on uptake and assimilation of nitrogen from nitrate and ammonium sources may account for the poorer performance sometimes observed when field-grown tobacco is fertilized with ammonium. To assess the effect of increasing root-zone acidity on the relative ability of tobacco plants to utilize these two nitrogen sources, 6-wk old tobacco transplants ('Coker 139') were grown hydroponically for 8 days in complete nutrient solution containing 1.0 mM nitrate and then exposed to complete nutrient solution containing 1.0 mM nitrate or 1.0 mM ammonium for 28 days. Solution pH was maintained at 6.0 or 4.0. Plants were sampled at 6 to 8 day intervals to determine treatment effects on leaf initiation, leaf area and number, dry matter production and nitrogen content of plant tissues. Tobacco plants grown under nitrate nutrition acclimated to low root-zone pH and throughout the experiment had similar leaf area and number, photosynthetic rates and dry matter production at pH 4.0 and pH 6.0. In contrast, plants grown under ammonium nutrition at a solution pH of 4.0 showed severe inhibition of photosynthesis and growth after 13 days of exposure, and eventually died. However, if solution pH was kept at 6.0, plants grown with ammonium nutrition maintained comparable photosynthetic rates and yielded similar total dry matter and leaf area and number as plants grown with nitrate nutrition at either solution pH. Tissue samples collected during this experiment are being analyzed for total and nitrate nitrogen to determine the extent to which effects of pH on root function altered the relative ability of tobacco plants to assimilate nitrogen from nitrate and ammonium sources.

B. Effect of Nitrate Concentration:

Results from previous research associated with this laboratory have been used to validate a dynamic model for plant growth. To provide data to extend the structure of this model to include the effects of nitrogen availability on growth processes, 6-week old tobacco transplants were grown hydroponically for 7 days with 1.0 mM nitrate in a complete nutrient solution and then exposed to complete nutrient solution with 1.0 or 10.0 mM nitrate for 25 days. Plants were sampled at 3 to 4 day intervals to determine treatment effects on leaf initiation, leaf area and number, dry matter production and nitrogen content of plant tissues. Nitrate concentration had no significant effect on leaf area, leaf number, photosynthetic rate per unit leaf area, or dry matter production and distribution.

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Freeze-dried tissue samples will be analyzed for total and nitrate nitrogen to assess effects of different external nitrate concentration on nitrogen partitioning within the plant.

Monitoring of solution nitrate depletion with ion chromatography indicated a cyclic pattern of nitrate uptake on a per plant basis. Nitrate concentration substantially altered the amount of nitrate taken up per plant in any given day, with the greatest fluctuations in uptake occurring for plants grown in 10.0 mM nitrate. At this high nitrate concentration, nitrogen absorption apparently often exceeded demand for nitrogen in the plant to the extent that nitrate was periodically lost to solution rather than being stored within the root for subsequent reduction and/or translocation to the shoot. Cumulative depletion of nitrate from replenished solutions over the treatment period will be compared to nitrogen accumulation in the plant as measured by tissue analysis to validate the rates of nitrate uptake as calculated from solution depletion.

C. Effect of Nitrogen Stress:

Previous experimental results with soybean indicate that, at reduced levels of nitrogen in solution, plants have decreased nitrogen uptake by roots and decreased leaf expansion. Demand for carbon in the leaf sink declines and there is a shift in carbon partitioning from the leaves to the roots as a balance between root and shoot functioning is restored. In a follow-up study we investigated the response of tobacco plants to developing internal nitrogen stress and removal of that stress when grown under standard irradiance level (700 μmol m^{-2} sec^{-1}). Plants were also grown under reduced irradiance (350 μmol m^{-2} sec^{-1}) to determine how a change in aerial environment, which reduces photosynthesis and affects carbon partitioning and demand for nitrogen, alters the interdependence of root and shoot functioning in response to nitrogen stress and removal of that stress. Six-week old tobacco transplants were grown hydroponically with 1.0 mM nitrate in a complete nutrient solution until the 7- to 8-leaf stage. Plants were then exposed to complete nutrient solution with 1.0 or 0.0 mM nitrate for 8 to 9 days when photosynthetic rates of the youngest mature leaves of stressed plants were 40 to 45% of values for the nonstressed plants. Nitrogen stress was then relieved for half of the plants by transferring seedlings back into a 1.0 mM nitrate solution for 19 to 21 days, while the other half remained in a 0.0 mM nitrate solution. Plants were sampled at 5 to 7 day intervals to determine treatment effects on leaf emergence, leaf area and number, dry matter production and nitrogen content of plant tissues. When grown under a lower irradiance level, photosynthetic rate of seedlings that received 1.0 mM nitrate continuously was 70% of that for tobacco plants grown under high irradiance. Thus, the decrease in photosynthesis under low irradiance would be expected to affect nitrogen uptake even prior to imposition of a nitrogen stress treatment. Under low irradiance, nitrogen stress had a greater effect on leaf emergence and expansion than seen for plants grown under higher irradiance. Plants continously exposed to nitrogen stress, under low irradiance had final total leaf areas only 6% of the nonstressed plants, while leaf areas of continuously stressed plants under high irradiance were 14% of that for the nonstressed plants. Over the recovery period, photosynthetic rate of nitrogen stressed seedlings returned to the rate of plants that received 1.0 mM nitrate continuously under both irradiance conditions. However, renewal

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of leaf expansion and emergence was delayed under low irradiance, indicating that the balance between carbon utilization and uptake of nitrogen by the root and carbon and nitrogen utilization by the shoot wasn't restored as rapidly as under high irradiance conditions. Plant tissue samples from this study are being analyzed for total and nitrate nitrogen and soluble carbohydrates to determine the effects of nitrogen stress and removal of that stress on partitioning of nitrogen and soluble carbohydrates within plants grown under high and low irradiance.

D. Effect of Leaf Water Stress:

Tobacco seedlings were transplanted into 9-liter pots filled with sand and placed in a growth room in the phytotron. Emitters from an automatic watering system were placed in each pot. Plants were divided into three groups. For the nonstressed group, the frequency and volume of water applications were adjusted to maintain leaf water potentials during a 3 week period between levels of -0.2 to -0.4 MPa; for the slightly stressed group, applications of water were adjusted to maintain leaf water potentials between -0.6 and -0.7 MPa; and for the moderately stressed group, applications were adjusted to maintain leaf water potentials between -0.7 and -0.8 MPa. Two experiments were conducted: one at high irradiance and one at low irradiance. Net photosynthetic rates per unit area of leaves under high irradiance were reduced 18% and 23% relative to nonstressed plants in the slightly and moderately plants, respectively, and under low irradiance, were reduced 15% and 25%. The effect of leaf water stress on leaf expansion was greater than the effect on net photosynthetic rate per unit leaf area. Under high irradiance, leaf expansion was reduced 27% by the slight stress and 37% by the moderate stress. Under low irradiance, the reductions in leaf area were 29% and 55% by slight and moderate stresses. These reductions in photosynthetic activity (or the product of reductions net photosynthetic rate per unit leaf area and reductions in leaf expansion) caused by leaf water stress resulted in reductions in total dry matter accumulation by the plants. However, the reductions in dry matter accumulation were not as great for roots as for leaves and stems. In fact, under high irradiance, dry matter accumulation in roots of slightly stressed plants was the same as for nonstressed plants. These results indicate that when leaf water stresses occur, plants acclimate by partitioning a greater proportion of the photosynthate for root expansion.

II. Graduate Students:

Celia L. Cannon (M.S.)
Tom C. Granato (Ph.D.)
June Taeg Lim (Ph.D.)

III. Postdoctoral Fellows:

Dr. Leslie C. Tolley

V. Manuscripts Accepted for Publication:

Tolley, L. C. and C. D. Raper, Jr. 1985. Cyclic variations in nitrogen uptake rate in whole plants. Plant Physiol. 78: in press.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

V. Manuscripts Accepted for Publication:

Wann, M., and C. D. Raper, Jr. 1985. Long-distance transport in phloem - A mathematical simulation model. In D. Newman and K. Wilson (eds.) Model building in plant physiology/biochemistry. CRC Press, IBc., Boca Raton, Florida.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

Title: NCO 3987 Cultural, Nutritional and Varietal Investigations with Burley Tobacco

Project Leader: J. E. Shelton

I. Summary of Research:

This project has the following objectives:

A. To evaluate methods of distinquishing differences in P and K requirements of soils with differing chemical and physical characteristics.
B. To correlate information from objective A with yield and quality of burley over a wide range of soil P and K levels and soil management systems.
C. To investigate the use of urea as a N source for burley under different soil management systems.
D. To evaluate advanced breeding lines and commercial varieties of burley tobacco through cooperation in the "Regional Burley Tobacco Variety Testing Program."

Field investigations to gain information on objectives A and B were established in 1984. Yields and quality were highly dependent upon soil type, and nutrient level. Soil samples have been taken for detailed laboratory studies on methods of determining the phosphate requirements on various soil types at given soil test results. This is a relatively long time evaluation and limited information has been obtained at this time.

In evaluating urea as a possible N source with Vorlex and Terr-O-Cide 30 fumigants in 1983 and with Vorlex in 1984 at N rate of 112, 224 and 448 kg N/ha plant survival following transplanting was lower at the highest N rate regardless of source. The highest plant mortality occurred at the 488 kg urea- N/ha when fumigated with Vorlex and grown on plastic. At the lowest N rate of 112 kg N/ha the yields were lowest with $CaNO_3$; with NH_4NO_3 being intermediate with no significant difference between NH_4NO_3 and urea. At the higher N rates there were no significant differences in yield regardless of fumigant. Total alkaloid content of cured leaves was highest with the Vorlex plus plastic fumigation treatment but fumigation with Vorlex without plastic did not affect alkaloid content. Total alkaloids were not affected by rate or source of N.

The"Regional Burley Tobacco Variety Evaluation Committee" met in Kingsport, Tennessee on Feb. 26. Of the 22 entries included in the small plot test, two were standard varieties, six had previously been approved for farm-test entry, one was withdrawn by the breeder due to segregation and the following met all criteria for advancement; Greenville 131,132, 135 and 136; Va 507 and two North Carolina lines, NC-137 and NC-71. All other entries failed due to a high nicotine content.

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On the On-Farm test which includes lines previously passed by the committee in small plot tests, Rogers 81-58-3 was rejected due to high nicotine. Va 503, Va 510, Rogers 81-62-1, Coker 82-161-2E and Coker 83-3Y2 passed all requirements except smoke test, the results of which were not available at this time, and were tentatively approved (dependant on smoke test) for release as varieties at the breeders discretion. However, the committee recommended to the breeder that Coker 83-3Y2 not be released due to extremely high virus susceptibility.

VII. Papers Presented at Professional Meetings:

Shelton, J. E. 1985. Effect of Bedding on Response of Burley Tobacco to Nitrogen Fertilization. The 31st Tobacco Workers Conference. Jan. 7-10, 1985. Pinehurst, NC.

Shelton, J. E. and P. B. Shoemaker. 1985. Effect of Soil Fumigation on Urea as an Nitrogen Source for Burley Tobacco. The 31st Tobacco Workers Conference. Jan. 7-10, 1985. Pinehurst, NC.

IX. Acknowledgements:

Appreciation is expressed to Dr. P. B. Shoemaker for his cooperation in the fumigation research and to Hugh Russell and P. D. Tate for their invaluable assistance in conducting these studies.

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Source: https://www.industrydocuments.ucsf.edu/docs/ssyk0000

Title: NC 3819 Cover Crop Evalaution and No-Till Crop Production on Mountain Soils of North Carolina.

Project Leader: G.D. Hoyt

I. Summary of Research:

This project has the following objectives:

A. To identify winter covers which best suit climatic and agronomic conditions of the mountain region.

B. To determine the effect cover crop residues have on conservation tilled burley tobacco yields.

C. To establish conservation tillage for burley tobacco production in the mountain region on soils subject to erosion.

D. To determine optimum fertilizer source and placement for conservation tilled burley tobacco.

A tobacco management system was established in 1983 and 1984 to develop cultural practices which would decrease erosion, increase water infiltration, retain soil moisture, and provide comparable yields to conventional tillage. This system utilized a fall planted cover crop that was spring killed, strip tilled with a subsoiler-coulter combination and planted with regular transplant equipment. Cover crops were established in the fall of 1983 with previous summer crop residue and fertilizer P, K, and limestone incorporated at cover crop planting. Previous to tobacco setting, the various cover crops were sampled for biomass and then killed with glyphosate. Approximately seven days later a sub-soiler-coulter combination (Bush-hog Ro-till) was run through the field to establish rows. This equipment provided 6 to 10 inch wide tillage of the soil surface. Preplant - surface applied pesticides and required nitrogen were applied at this time.

Yield results for 1984 were very similar to those in 1983. At both experimental locations, burley tobacco production was greatest where winter residues had accumulated the thickest mulches. Cover crop residue affected burley yields in the following order at the Mountain Research Station: Rye > hairy vetch > crimson clover > bare soil > barley > cultivated soil > mulch > ryegrass. At a location in Buncombe Co., the residues again played an important part in resultant tobacco yields. Residues in these conservation-tilled field plots gave the following order of burley tobacco production: A. winter peas > rye > mulch > wheat > ryegrass > barley > cultivated soil. A quality index rating was calculated from the graded tobacco, but at both locations no significant differences were found among the residue treatments. Soil moisture readings taken throughout the experiment showed higher levels of moisture in plots with thick residues, thus a potential yield advantage for this non-irrigated test. Soil temperature measurements revealed cooler soils with these residues, but overall temperatures warm enough to provide adequate root growth.

IV. Publications:

Hoyt, G.D. 1984. The effect of cover crops on strip-till vegetable and tobacco production. Proc. Soil Science Soc. North Carolina. 27:10-20.

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V. Manuscripts accepted

Hoyt, G.D. and W.L. Hargrove. 1985. Legume cover crops for improved crop and soil management in the southern U.S. HortScience 20:In Press.

VII. Papers presented at professional meetings:

A. Legume cover crops for improved soil and crop management in the Southern U.S. Invited paper before the Interplanting in Horticultural cropping Systems Symposium, American Soc. for Hort. Sci. at Vancouver, B.C. Canada, August, 1984.

B. Winter Cover Crops for No-till Vegetable and Tobacco Production. Presented before Division S-6, Amer. Soc. of Agronomy at Las Vegas, Nevada, November, 1984.

C. Winter cover crops for no-till tobacco production. Presented before the Burley Section, 31st Tobacco Workers Conference at Pinehurst, N.C., January, 1985.

IX. Acknowledgements:

I wish to thank Mr. Anthony Cole for his excellent technical assistance on this project.

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TOBACCO LITERATURE SERVICE

Annual Report for the Calendar Year 1984

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		1983	1984	Change
I.	TOBACCO ABSTRACTS.			
	Abstracts published	1,924	1,357	− 567
	Publications searched	5,837	5,580	− 257
	Average per month	503	465	− 38
	Distribution			
	Domestic:(207)			
	Exchange	49	48	− 1
	Complimentary	106	53	− 53
	Subscriptions	103	106	+ 3
	Foreign:(318)			
	Exchange	139	140	+ 1
	Complimentary	28	27	− 1
	Subscriptions	169	151	− 18
	Total	594	525	− 69
	Copies per year	3,564	3,150	− 414
II.	TOBACCO REPRINT SERIES.			
	Reprints published (#689-706)	11	17	+ 6
	Recipients	162	164	+ 2
	Copies distributed	1,782	2,975	+ 1,193
III.	BIBLIOGRAPHIC INFORMATION SERVICE			
	Requests served	1,100	1,050	− 50
IV.	PHOTOCOPY SERVICE.			
	Tobacco Staff	6,049 p.	6,074 p.	+ 25
	Paid orders	1,072 p.	1,067 p.	− 5

V. OTHER PUBLICATIONS DISTRIBUTED.

"Recent Advances in Tobacco Science".	59 copies
"Tobacco Reprint Series; 640 Publications by the Tobacco Staff, North Carolina State University 1954-1981" by Joseph A. Weybrew.	80 copies
"Theses on Tobacco. I. North Carolina State University. II. United States Universities" by Carmen M. Marin.	200 copes
"Tobacco Literature, a Bibliography" by Carmen M. Marin	35 copies

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ADDENDUM

Listed below, by department, are research activities which received supplemental funding from the North Carolina Tobacco Foundation, Inc. for the period July 1, 1984 through June 30, 1985.

Activity	Investigator(s)
Biochemistry	
Development of Ripening Agents for Tobacco	E. C. Sisler
Biological and Agricultural Engineering	
Development of Fourier Transform NIR Instrumentation for Rapid Chemical Analyses of Tobacco	W. F. McClure W. W. Weeks F. G. Giesbrecht
Stress Physiology of Tobacco Seedling Development	S. C. Mohapatra
Curing Conditions for Quality Tobacco and Fuel Efficiency	R. W. Watkins
Burley Mechanization	C. W. Suggs
Microcomputer Software Development	R. S. Sowell
Botany	
Cytological and Anatomical Changes in the Apical Meristem of Tobacco During the Transition to Flowering	J. F. Thomas
Molecular Characterization of Genetic Variability in Anther Derived Dihaploids of Tobacco	J. P. Miksche S. S. Dhillon E. A. Wernsman
Crop Science	
Improving and Automating the Analytical Methodologies Required on Experimental Tobacco	W. W. Weeks
Investigations of Haploid Methodologies for Tobacco Improvement	E. A. Wernsman

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Activity	Investigator(s)
Crop Science (continued)	
Genetic Studies of Resistance to Tobacco Vein Mottling, Tobacco Etch Virus and Potato Virus Y on Burley Tobacco Using in vitro Methodologies	R. C. Rufty
Chemical and Smoke Panel Comparison of Popular Tobacco Varieties Grown from 1940-1983	W. W. Weeks J. F. Chaplin
Remobilization of Nitrogen During Growth and Senescence of Flue-Cured Tobacco	R. C. Long
No-Till Tobacco	A. D. Worsham W. D. Smith
Isozyme Patterns in Haploid and Double Haploid Populations of Tobacco	V. A. Sisson
The Effect of CO_2 Enrichment on Growth and Quality of Tobacco	T. W. Rufty, Jr.
Interspecific Hybridization in Nicotiana	S. M. Reed
Qualitative and Quantitative Analysis of the Sucrose Esters of Tobacco	D. A. Danehower
Economics and Business	
Explanation of Size and Structure in Tobacco Farming: Including an Analysis of Potential Changes	D. A. Sumner
Entomology	
Population Dynamics of the Tobacco Flea Beetle and its Effects on Tobacco Yield	E. P. Lampert
Continued Development of Improved Sampling Methods for Insect Abundance in Tobacco for Pest Management Programs	E. P. Lampert
The Use of Feeding Deterrents to Protect Tobacco from Wireworm Damage	F. Gould P. S. Southern
Soil Insects Associated with No-Tillage and Cover Cropping Practices in Tobacco	G. J. House

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Activity	Investigator(s)
Entomology (continued)	
Behavioral Responses of Female Tobacco Budworm Moths to Cuticular Diterpenes from Green Tobacco Leaves	D. M. Jackson
Entomology (Pesticide Residue Research Laboratory)	
Residue Studies with EBDC Fungicides on Flue-Cured and Burley Tobacco	T. J. Sheets
Residues of Prime+ on Flue-Cured Tobacco	T. J. Sheets H. Seltmann
An Automatic Sampling and Injection System for a New High Performance Liquid Chromatograph	T. J. Sheets
Genetics	
Genetic Modification of Tobacco	D. F. Matzinger
Plant Pathology	
Survival of P. solanacearum in Soils, Crops and Weeds and Predisposition of Tobacco Plants to Granville Wilt by Nematodes	E. Echandi
Epidemiology of Tobacco Blue Mold	C. E. Main
Epidemiology of Tobacco Black Shank Disease	H. D. Shew
Alternative Fungicides and Strategies for Blue Mold Control on Burley Tobacco	P. B. Shoemaker
Tobacco Blue Mold - Identification of Pathogenic Races	H. W. Spurr, Jr.
Transfer of Resistance Genes from Nicotiana repanda to Nicotiana tabacum by Protoplast Fusion	M. E. Daub
Cross Protection with Potato Virus Y and Tobacco Mosaic Virus Inhibitor Studies on Flue-Cured Tobacco	G. V. Gooding, Jr.
Identification of and Breeding for Resistance to Tobacco Vein Mottling and Tobacco Etch Viruses on Burley Tobacco	G. V. Gooding, Jr.

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Activity	Investigator(s)
Plant Pathology (continued)	
Control of Flue-Cured Tobacco Diseases by Means of Cultural Practices, Varietal Resistance and Chemical Application	N. T. Powell
Breeding, Genetics and Biological Control Studies on Granville Wilt of Tobacco	G. R. Gwynn H. W. Spurr, Jr. E. Echandi
Soil Science	
Site Specific Subsoil Requirements	G. S. Miner M. J. Vepraskas
The Influence of Tillage and Cover Crop Management on Burley Tobacco Production	G. D. Hoyt
Soil Management Practices for Improved Water Conservation, Erosion Control and Tobacco Production	C. B. McCants G. R. Bathke H. P. Denton
Determination of the Optimum P & K Requirements for Burley Tobacco Production, Quality and Fertilizer Efficiency	J. E. Shelton
Nitrate Assimilation by Tobacco as Regulated by Carbohydrate Status	R. J. Volk T. W. Rufty, Jr.
Whole-Plant Dynamics and Regulation of N Uptake by Roots	C. D. Raper, Jr. Leslie C. Tolley
Sociology and Anthropology	
Tobacco Farming as a Component of Agricultural Structure: Its Relationship with Social Well-Being	R. C. Wimberley
General	
Director's Reserve for Emergencies and Reserve Research Assistantships	W. H. Johnson

Please keep in mind that the information presented in this annual report is NOT FOR PUBLICATION but merely represents preliminary findings which may later appear in published form. We trust that you will find the information presented herein of interest and value.

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